

The profits of being bankrupt

The Manville Corporation is using the US bankruptcy laws legally but too cleverly to avoid costly damage suits from asbestos workers. It needs help from Congress.

MANVILLE Corporation is by all accounts the most profitable bankrupt company in history. One year ago this week, Manville filed for bankruptcy to the astonishment of the financial world and the outrage of thousands of asbestos workers who were suing or preparing to sue the former asbestos-manufacturing giant for the damages they suffered from breathing asbestos fibre. Under the bankruptcy court's protection, Manville has freed itself from all immediate obligations, has blocked the filing of new lawsuits against it (which were coming in at the rate of 400 per month at the time it declared bankruptcy) — and has accumulated a phenomenal pile of cash and securities. The latter is probably not surprising in light of the fact that Manville was a profitable enterprise even as it was declaring itself bankrupt (operating income of \$43 million during the first six months of last year), and has become all the more profitable with its debt obligations frozen. Still, it is sobering to discover just how well the company is doing. Its holdings of cash and marketable securities have grown to \$233 million from \$27 million a year ago. And its operating income for the first six months of this year is nearly double last year's level.

The outcry that greeted Manville's bankruptcy filing — specifically, the accusation that a financially healthy company was abusing the bankruptcy process to duck its legal obligations to asbestos victims — has not penetrated very deeply into the company's apparently thick hide. The company has already missed six court-ordered deadlines for filing a reorganization plan, as required by the bankruptcy law, and is not expected to meet the latest deadline of 15 September. The company's vice-president was in fact quoted in the *New York Times* last week as saying that it might take two years for a plan to be worked out. The company cannot complain if its critics conclude that Manville's chief concern is prolonging to the limit its stay in legal never-never land.

The company's plan for reorganization foresees splitting the company into two parts: one will contain the money-making building-products business; the other will do nothing but handle asbestos claims. The plan deserves an award for sheer audacity if nothing else.

The issue of Manville's culpability — or, more to the point, its liability — is, to be sure, far from cut and dried. The apportionment of liability is largely a matter of common law — that is, judicial precedent — and over the past 60 years in the United States the courts have placed dramatically increased responsibility for the safety of a product on the manufacturer. Until the 1920s, product liability claims had to be based on a warranty. A landmark suit against Buick changed that when the court ruled that negligence on the manufacturer's part was sufficient grounds. More recently, courts have held manufacturers to a standard of strict liability in some cases, which drops even the negligence requirement. Strict liability is based in part on the legal theory that the manufacturer is best able to bear the social cost of damage done, regardless of who is at fault.

Manville's position is complicated by the role of the US Government during the Second World War in directing the manufacture of asbestos and its use in shipbuilding. Roughly half of the 20,000 claims filed against Manville involve workers at wartime shipyards that were either owned by the government or under direct government control or contract, and Manville recently sued the government to recover the costs of some of its settlements with

these workers. The company has produced some damning government documents from the period which appear to support its case that the government was aware of an asbestos hazard at these facilities.

The resolution of this issue is unquestionably a matter for the courts, and Manville is well within its rights and the bounds of propriety to seek, through a lawsuit against the government, a judicial determination of where the liability lies. It would likewise be appropriate for industry as a whole to seek a legislative declaration of how, generally, liability is to be apportioned in a world that we should know by now cannot be risk-free. The social policy question of who should and is best able to bear the costs of injury to workers or consumers is indeed not one that should be left by default to the courts.

The tactical use of bankruptcy protection by a profitable company is quite a different matter. By continuing to hide under the protection of the court and by dragging the proceedings on as long as possible, Manville's appeal for a judicial finding of the government's share of liability becomes hypocritical at best. And in the long run, such hypocrisy can only harm the prospects for a much needed statutory adjustment of the assignment of risk. □

Star wars treaty?

The Reagan Administration has been too quick to reject the Soviet draft of a treaty on space weapons.

SHOULD there be a formal treaty banning the use of bows and arrows for military purposes? Or one that would for all time outlaw the use of telehypnotism (long-distance mind control on which dedicated teams of parapsychologists are working in secret) as a means of subverting political leaders' resolution? And how should responsible governments respond when fellow governments submit draft treaties for these purposes to the United Nations? Luckily, several centuries of diplomacy have provided all the language needed to deal with awkward questions such as these. Unhappily, none of this was evident in the sour response from Washington to Mr Yuri Andropov's draft treaty on space weapons (see page 3).

Mr Andropov's proposed treaty is, of course, serious — for which reason it might have been expected to have evoked a serious response. As things are, the only feasible applications of star wars technology are in the destruction of other people's military satellites, presumably at the outset of hostilities of some kind. President Ronald Reagan has, however, been advocating research and development aimed at the unfeasible goal of perfect defence against ballistic missiles. Mr Andropov's draft treaty is a valid, if cheeky, counter-move. The Reagan Administration seems not to appreciate that dismissing it, with hints that the Soviet Union is in any case "ahead" in the development of antisatellite weapons, is a false move. A workable treaty on space weapons would be well worth having, for these devices are neither bows and arrows nor telehypnosis. The assertion is also false that negotiations on a space treaty would distract attention from the more important negotiations of missiles (intermediate and long-range) still (mercifully) dragging on in Geneva. The truth is probably the opposite. Any treaty, on almost any subject, would help people to sustain hope that something will come from the Geneva negotiations. □



Yellow rain

Contradictory accounts gathered in Laos

Washington

Two Americans who were allowed to conduct on-the-scene investigations of reported "yellow rain" attacks against Hmong villagers in Laos are about to publish findings that raise considerable doubt about US State Department allegations of the use of chemical weapons in the region.

In January and February this year, Jacqui Chagnon and Roger Rumpf visited several areas that have figured in US reports of yellow rain attacks. They are fluent in Laotian, having worked as field directors for the American Friends Service Committee in Laos from 1978 to 1981. During that time they were in a party of international aid representatives who toured the Phu Bia mountain area, a centre of Hmong resistance, at the same time (March 1979) that chemical attacks were going on there according to State Department charges.

Their interviews with Hmong villagers and Lao government officials this year appear to contradict both in detail and in general impression, the US charges that the Laotian government, with Soviet support, is waging genocidal war against the Hmong and is using chemical weapons to do so.

The US charges are based on interviews with refugees in Thailand, on intelligence reports and on chemical analyses of blood samples which show the presence of trichothecene mycotoxins, the fungal toxins that the United States says are being used as a chemical weapon. However, neither US officials nor a United Nations scientific team have been allowed inside Laos to investigate the reports.

Chagnon and Rumpf were permitted to travel through the countryside and conduct interviews, often in the presence of government officials. The most striking of their findings, which are soon to be published in a specialist journal on South-East Asia*, is the mixture of story-telling, rumour, myth and contradiction that worked its way into accounts of yellow rain told to them by Hmong villagers; striking, too, is the fact that many of the accounts of yellow rain or droplets and associated deaths and illness came from Laotian government officials.

A typical instance occurred in Ban Done, a Hmong village that has been cited by many Hmong refugees in Thailand as the site of chemical attacks. In Ban Done, Chagnon and Rumpf interviewed a government official, Xua Chang Her, a Hmong who related how he saw yellow "fog or smoke" killing people, buffalo and rice in several villages in 1982. Similar stories came from villagers who had fought with Vang Pao; none, however, connected the

phenomenon with aircraft flying over or even with warfare. Several contradictory accounts (some of the contradictions appearing in the story of a single teller), dated the poisoning to December 1981 or September 1982.

In another village, yellow rain stories — again brought up by a local Hmong leader who sided with the government — were vigorously affirmed by everyone except the local medic, who said the ills the villagers ascribed to poisoning were in fact the result of cerebral malaria, a prevalent problem in the region. Chagnon and Rumpf report that none of two dozen foreign relief workers they spoke to who had travelled throughout the Hmong areas had seen any evidence of "yellow poisons".

Although they avoid drawing any firm conclusions about the use of chemical

weapons or the nature of yellow rain, Chagnon and Rumpf suggest that more cross-checking within Laos of refugee stories and intelligence reports is necessary. They say that they were told by a vice-minister of the Laos foreign ministry that Laos would now consider allowing a private, impartial team of scientists into the country to investigate the yellow rain charges, and that a similar comment was made by the prime minister to a prominent European diplomat. The government apparently continues to oppose any official delegation, even from the United Nations, and the problem has become one of organizing a private delegation and raising support. Chagnon estimates the cost to be at least \$50,000–\$60,000. The European diplomat who received the prime minister's assurances that such a delegation would be welcome reportedly tried to assemble a group of scientists in Europe and the United States, but has since been posted outside South-East Asia.

Stephen Budiansky

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Computers

NSF pressed on supercomputers

Washington

TEN supercomputer centres should be established in the next three years to improve US computational facilities for academic research, according to a draft report of a working group of the National Science Foundation (NSF).

In justification of the \$200 million it wants NSF to spend on the centres, the group says that "Important science is not being done in this country due to lack of access to adequate supercomputer capacity." The report adds: "Even these investments would not provide computing facilities for academic researchers comparable to those at a typical national or industrial research laboratory."

The group reports several trends in the 1970s that led to the present neglect of large computing capabilities at universities. NSF itself stopped supporting campus computer centres in 1972; this support had allowed a number of universities to obtain the "supercomputers" of the period — IBM 370s and CDC 7600s. Meanwhile, individual departments and groups began acquiring their own mini and microcomputers, a move spurred on by the refusal of most federal funding agencies to support the full cost of purchased computer time in research grants. And, the report notes, many campus computer centres fell into "disrepute" among scientific users because they gave priority to small jobs and administrative and educational tasks.

According to the report, existing supercomputer facilities in the United States are either designed around a single

discipline (such as the CRAY-1 parallel-processor computers at the National Center for Atmospheric Research and the Department of Energy's Magnetic Fusion Energy Computer Center) or prohibitively expensive (typical charges running at \$1,000–2,000 per hour) or not configured (in hardware or software) for research uses. One American astrophysicist is said to have had to establish a collaboration with scientists in West Germany in order to gain access to an appropriately configured supercomputer.

Some critics of the NSF group's gung-ho attitude towards supercomputers complain that the supercomputer is a solution looking for a problem. However, after the NSF group convened a workshop in May to solicit suggestions for possible uses of supercomputers, the group was able to make a convincing case for such machines for several applications, including:

- quantum mechanical calculations of reactions of complex molecules;
- modelling of the formation of stars;
- modelling dynamic magnetic systems;
- fine-resolution modelling of the atmosphere and oceans.

The working group's report also recommends vastly increased support for the purchase of mini and microcomputers by individual groups and departments — \$240 million over three years, up from the currently planned \$170 million — with special emphasis on engineering, physics, atmospheric research and computer science.

Stephen Budiansky

UK asbestos

Down and going out

A NUMBER of measures aimed at reducing exposure to asbestos fibres in the workplace were announced by the British Health and Safety Commission last week. Allowable airborne concentrations for two types of asbestos will be reduced from next August and new licensing regulations have been proposed to control work on asbestos insulation or coating.

In a statement issued after the meeting the commission said "it is clear from medical reports now available that there is no longer any medical doubt about the need for controls to be as tight as possible in workplaces where asbestos is present".

The new fibre concentrations are close to the limits for accurate measurement using widely available equipment and will hasten the decline in the use of asbestos in manufacturing industry. The new limit for chrysotile (white asbestos), the least dangerous of the common forms and the only type widely used in British manufacturing, is 0.5 fibres per millilitre of air, half the previous limit. Some products, such as asbestos cement, can easily be manufactured without exceeding this limit. But for woven products, such as fireproof clothing, control can be impossible, and companies are then obliged by law to ensure that protective respiratory equipment is provided and worn.

The real pinch of the decisions reached last week will be in the area of demolition and maintenance work, which is where the highest levels have been recorded and also where engineering control methods are most difficult.

Amosite (brown asbestos) will in future be classed with crocidolite (blue asbestos) and be banned from importation or use in industry. Both, however, are still present in the insulation of many buildings and can be released in dangerous levels when the buildings are stripped or demolished. The commission has proposed new licensing regulations to eliminate that; the proposals are to be considered by government.

The commission welcomed two European Community directives agreed in June which require a formal notification and medical surveillance system.

Existing asbestos regulations apply only to the workplace, and the commission has been under strong public pressure to introduce comparable controls for the general environment. To explore that possibility, it is to initiate discussions with other government departments.

It has also to set up an asbestos industry working group which will prepare guidance on methods of engineering control and the use of protective equipment. Two of the reports the commission had before it last week were severely critical of the way existing guidance is implemented in factories, where guidelines are widely disregarded partly because of the discomfort caused by

wearing protective face masks for long periods.

As the use of asbestos substitutes becomes more widespread, the commission will have to consider expert opinion that it

is the physical size and shape of asbestos fibres, rather than their intrinsic chemical nature, which is responsible for their carcinogenic properties and which gives rise to asbestosis. Although most substitute fibres are much larger than asbestos fibres, and therefore less dangerous, some ceramic fibres used in insulation work might also be carcinogenic.

Tim Beardsley

Satellite weapons

Soviets propose space ban

THE Soviet proposals for a treaty banning the "use of force in space and from space with regard to Earth", tabled for consideration at the next session of the United Nations General Assembly, comes at a somewhat inopportune moment for the United States, which is about to test a new antisatellite missile. The timing is not necessarily deliberate. August is the appropriate time for putting down motions, and it is now two years since the previous Soviet proposals were tabled.

The existing treaties banning the militarization of space (1967 and 1979) refer only to "weapons of mass destruction". The 1981 draft would have outlawed all kinds of weapons in space but made no specific reference to antisatellite devices, upon which the new draft specifically concentrates.

The Soviet Union, ironically, has itself carried out a number of hunter-killer satellite tests during the past sixteen years, the launches being carried out under the multi-purpose Cosmos programme. The original fast fly-by interceptions were replaced in 1980-81 by the more sophisticated co-orbital flight-plan. Although the treaty, as drafted, would make it incumbent on the Soviet Union to "eliminate" any existing antisatellite

devices, signing it would still leave the Soviet Union with a considerable file of test results in its military archives.

Apart from the vexed issue of verification, which the Soviets propose should be carried out using only "national technical facilities", the new draft contains several controversial points.

Clause five, for example, forbids the use of any manned spacecraft for military purposes. Since a major task of orbital manned missions is resource reconnaissance photography, what happens if a team observing, say, global crop disease patterns happens to record evidence of military preparations? Clause two forbids the use of space objects "as a means of hitting targets on Earth, in the atmosphere or in space", which could hinder geodesic research based on the accurate observation of satellite orbits since such data are also essential for the programming of intercontinental ballistic missiles. The proposed treaty would apparently also forbid signatory states from owning any means of destroying their own satellites in orbit despite international concern over the unscheduled re-entry of radioactive power packs from rogue or re-routed satellites, to say nothing of the danger of debris re-entering over a populated area.

Vera Rich



Ground stations for monitoring orbital satellites, established under the Intercosmos programme. (From Vestnik AN SSSR no. 5, 1983)

Agent Orange

Survey spells out hazards

Washington

EXPOSURE to dioxin-contaminated herbicides does not seem to have caused any unusual health problems in 85,000 veterans of the Vietnam war studied by scientists at the Veterans Administration (VA). In a paper presented at the American Chemical Society meeting here earlier this week, Dr Alvin Young said that the "wide variety of health problems" that do appear in the group are typical of a male population that is growing older.

Earlier this summer, the Air Force released preliminary findings of an epidemiological study of 1,200 veterans who handled the herbicides daily in Vietnam; no excess mortality was found in this group as compared with a group of similar veterans who did not handle herbicides. Results of the more significant morbidity study of the group are to be released in October.

Although the VA survey might have detected especially serious and widespread effects of herbicide exposure, it is not a very sensitive study. The 85,000 subjects were a "self-selected" group, veterans who presented themselves at VA hospitals with complaints that they attributed to possible exposure to herbicides in Vietnam. But fully three-quarters of the patients said they did not know if they had in fact been exposed; thus any problems actually appearing in exposed veterans would probably be statistically diluted in such a survey. The only disorder that was more common among the group than in a reference population of US males of similar age distribution was lym-

phomas; 20 cases appeared in the veterans group, compared with an expected 15 — a "barely" statistically significant difference, Young said.

Young also reported that a small number of analyses of adipose tissue samples (33 subjects) showed detectable amounts of dioxin in about half of both exposed veterans and non-exposed veterans. Most of the detectable levels were in the 5–15 parts per 10^{12} range; the highest concentration, in one exposed veteran, was 100 parts per 10^{12} .

VA is checking 550 human adipose tissue samples on file at the Environmental Protection Agency in an effort to determine the background level of dioxin in the US population. Dioxin (specifically 2,3,7,8-tetrachlorodibenzo-*p*-dioxin) is an essential contaminant of the herbicide 2,4,5-T, which was widely used on farms and ranches in the United States until the 1970s, and which, as a component of Agent Orange was heavily applied to forests in Vietnam in the US defoliation campaign designed to deny cover for the enemy.

The morbidity results of the Air Force epidemiological study should provide the first substantial evidence for or against claims of chronic injury associated with dioxin exposure in veterans. Veterans have filed over 17,000 claims for disability compensation with VA in connection with Agent Orange exposure which the government has so far refused to pay, arguing that only chloracne — a skin rash — has been conclusively associated with dioxin. A

much larger epidemiological study of 30,000 veterans, which will provide more definitive answers, has been slow to get under way; pressure from veterans and veterans' groups such as the American Legion, which expressed doubts about VA's objectivity, led to this study being transferred from VA to the Centers for Disease Control early this year. The results are not expected before 1987.

Stephen Budiansky

Medicine

BMA at fringes

THE British Medical Association (BMA) has taken a tentative step towards acknowledging the validity of some unorthodox medical therapies by setting up a group to consider the feasibility of assessing their value. The group has asked for information on the types of therapy being offered and their practitioners' theories about how they may work.

Alternative medicine has been flourishing in Britain in recent years and the Prince of Wales, ex-president of BMA, recently argued that physicians should be more willing to consider the possible value of unconventional approaches to therapy. Moreover, a small poll published in BMA's *British Medical Journal* last month found strikingly favourable attitudes towards alternative therapies among a sample of 100 young general practitioners. More than half of the 86 respondents believed that acupuncture, hypnosis or homoeopathy could be useful methods of therapy. Eighty per cent wished to train in at least one of the alternative therapies listed, and more than 20 per cent already used one.

Commenting on the study, Dr Tony Smith, deputy editor of the *British Medical Journal*, said that doctors should be able to tell patients which alternative therapies have been evaluated by objective tests and which have not, applying the same standards as would be applied to new drugs. However, Professor James Payne of the Department of Anaesthetics at London Hospital Medical College and chairman of the BMA's investigating group, points out that many advances in medicine were made before the advent of the double-blind controlled trial. He believes other criteria might be more appropriate for some therapies.

There are few restrictions on the types of therapy a physician may offer in Britain, although guidelines issued by the General Medical Council, the profession's governing body, make it clear that a doctor carries the responsibility for his patient if he refers a case to a practitioner who is not medically qualified. The BMA does not have an explicit policy on alternative therapies but if first the association's board of science and education and then BMA itself accept the investigating group's report, a register of approved therapies might result.

Tim Beardsley



The Zoological Society of London has expressed alarm at the possible consequences of the war in Northern Chad to the animal populations in the area. As well as the inevitable human casualties, two endangered antelope species are likely to suffer — the scimitar horned oryx and the addax. The last survivors in the wild are found around the border between Chad and Nigeria. A herd of scimitar horned oryx is established at Marwell Zoological Park in Hampshire and there are plans to restock the wild habitat.

Canadian technology

Slimmer but more weighty

Toronto

FACED with a predicted loss of two million jobs by 1991 as a result of technological change, the Canadian federal government has set about beefing up the way in which it gets its scientific advice, even if at first sight it appears to be doing exactly the opposite.

The federal Department of Science and Technology is having its staff cut from 170 to about 90 people — but both officials concerned with the reorganization and the independent, though government-funded, Science Council are convinced that it will be a change for the better.

A key change is that Dr Louis Berlinguet, who became the permanent head of the department on 1 June, is also to be the government's chief scientific adviser, a post that previously did not exist in Canada. In that role, Dr Berlinguet, a biochemist with extensive experience both in research administration and government, will sit on key cabinet committees and will have instant access to the decision makers.

Paradoxically, the streamlining is intended to give the Department of Science and Technology the weight it lacked in the past in providing government with high-level strategic planning advice on scientific and technological matters and to meet criticisms that the government's approach to fostering the growth of high-technology industries lacks an overall strategy.

The changes come some months after Mr

Donald Johnston, a minister with combined responsibilities for economic development and science and technology, announced a federal "technology initiative". Almost \$100 million Canadian dollars is to be spent over the next two years on government projects, including \$22 million for a national biotechnology strategy and \$7.5 million to develop microelectronics testing stations. In addition, \$290 million has been earmarked for 15 new technology research and training centres.

But there are worries that in practice it may be difficult for the chief scientific adviser to double as head of the science department, reporting directly to Mr Johnston as his cabinet minister, while advising other ministers through the cabinet committees. Critics feel that to be truly effective, the chief scientific adviser should be independent of any single government department.

For its part, the Science Council is leaning towards the view that for Canada to build up high-technology industries to provide future jobs, the government may have to intervene more directly in promoting new projects than it has done so far. Languishing at an all-time low in the opinion polls, and with an election coming up next year, Prime Minister Pierre Trudeau's Liberal Government seems unlikely to be making the long-term choices.

Nicholas Hirst

Animal experiments

Researcher cleared on appeal

Washington

DR Edward Taub, the Maryland researcher who was convicted on animal cruelty charges last year following the "infiltration" of his laboratory by an animal-rights activist, has had his conviction overturned by the state's highest court. On 10 August, the Maryland Court of Appeals ruled that Taub was exempt from the state's anti-cruelty statute because the research at his Institute for Behavioral Research in Silver Spring was supported by federal funds.

The case had become a *cause célèbre* for opponents of animal experiments; Taub's conviction marked the first time that state anti-cruelty laws were successfully applied to a scientific researcher. The conditions in Taub's laboratory also became the subject of a congressional investigation which led to proposals for stricter federal laws governing the use of animals in research, and a National Institutes of Health investigation that led to the termination of Taub's \$200,000 grant for research which involved cutting the nerves of monkeys to model the effects of stroke.

Charges were first brought against Taub

when a member of a group called People for the Ethical Treatment of Animals went to the police with pictures he had taken of the laboratory while posing as an interested student willing to work for Taub as a volunteer. Prosecutors focused their case on the failure to provide adequate veterinary care for the monkeys.

Although many states exempt scientific research from their animal cruelty laws, Maryland is not among them, and lawyers are expressing bewilderment at the high court's ruling. "It's a terrible opinion", says Roger Galvin, assistant state's attorney for Montgomery County, Maryland. According to Galvin, the state legislature overwhelmingly rejected in 1982 a proposal to amend the law with a specific exemption for research. The prosecution is preparing a petition to the court to reconsider its ruling.

Taub is claiming exoneration in the court's decision, which he called a victory for freedom of scientific inquiry. Taub's laboratory, however, remains closed and he has no funds at present to continue his research.

Stephen Budiansky

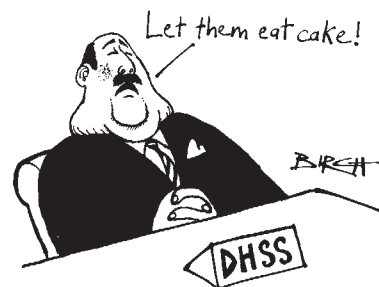
British nutrition

Report to surface at last

THE long-delayed report on nutrition in the United Kingdom, compiled by a working group of the National Advisory Committee on Nutrition Education (NACNE) will soon be made available to interested groups, in spite of the continued unwillingness of the Department of Health to give the report its approval.

The report, first drafted two years ago and kept out of the public eye until a few weeks ago (see *Nature* 14 July, p.103) recommends halving the average intake of sugar in Britain, a substantial decrease in dietary fat and a 50 per cent increase in fibre intake. These recommendations are based on publications from the Department of Health, the Royal College of Physicians and the World Health Organization.

The final version was recently approved at a meeting attended by the group's chairman, Professor Philip James of the Rowett Research Institute in Aberdeen, Professor Jeremy Morris, NACNE's chairman, and representatives from the British Nutrition Foundation and the Health Education Council. It was agreed that the report would be circulated this month to interested organizations "to make such use of it as they wish".



Surprisingly, the Department of Health was not represented at the meeting, although it has been extensively involved in proposing changes to the wording of various drafts. Very recently it unsuccessfully proposed a completely new preface for the report. At one stage the department apparently felt that NACNE had exceeded its remit; although it now accepts the need for the quantitative guidance that NACNE offers, it is still unhappy about some of the figures.

The department appears now to be dissociating itself from NACNE completely. A spokeswoman said forcefully last week "Professor James's report has nothing to do with us". James's group apparently started off as a subcommittee but its status has now changed to that of an "informal working group". NACNE's chairman, however, is still "very much hoping" that the department will endorse the report in due course, at least as far as supporting its interim goals.

Tim Beardsley

British Association

Scientists to be seen and heard

Brighton

EDITORS should give more prominence to scientists as people in their science features according to Professor John Maynard Smith of the University of Sussex, addressing a large audience at the annual meeting of the British Association. Scientists, for their part, should make a greater effort to be understood by the public, even at the cost of abandoning some of the qualifications they might ordinarily make to cover themselves.

While past generations learnt about science through the popular writings of active scientists, the young of today are more likely to become acquainted with science through a script written by a television producer or an article commissioned by a newspaper editor, he said. Although scientists cannot (and, indeed, should not) be the only interpreters of science to the public, there is a danger that editors may create controversy where none exists.

Maynard Smith claimed that some newspaper articles and television programmes had represented the gradualism *versus* punctuated equilibrium debate in evolution as though it were the last nail in the coffin of natural selection. The reality is that biologists are about as likely to abandon that theory as are chemists to abandon the atomic theory.

The blame for this sort of confusion usually lies with commissioning editors, who may have little understanding or affection for science, rather than with professional science writers. Some recent feature articles in the London *Times* had been commissioned from prominent scientists but their expertise lay in areas of science entirely different from that on which they were writing, said Maynard Smith, a biologist of distinction. "I can tell you that I have some doubts of my own about quantum physics. But I should hardly expect to be given a full page of a newspaper to write about them."

Although there are many excellent science programmes on television, the "voice-over" technique that is sometimes used denies scientists their identity as human beings. Professor Maynard Smith, whose face is well known to UK television audiences, drew an amusing caricature of many programmes: first of all the audience is treated to a shot of a white-coated junior scientist (probably a woman) pressing buttons on an incomprehensible machine, then a more senior scientist (probably a man, and probably not wearing a white coat) is shown sitting behind a desk getting very excited about something. Before he can explain himself an anonymous "voice-over" offers the producer's interpretation of what is going on.

Tim Beardsley

European Community

Counting the cost of agriculture

Brussels

RUNAWAY farm spending is the most pressing problem facing the European Community when it returns from its annual vacation in September. Until the problem is solved, the Commission's ambitious plans for spending on technology and scientific research cannot progress.

Agricultural spending — which gobbles up more than two-thirds of the Community's annual budget of 25,000 million ECU (1 ECU = £0.58) — has long been a point of contention, pitting countries with strong farm lobbies against those with weaker lobbies that are more disposed to change. What adds a touch of drama to the present situation is the fact that the predicted 30 per cent increase in farm expenditure this year will completely exhaust the Community's finances.

The failure of European farm policy and its system of open-ended production and export subsidies is illustrated by the present state of farm surpluses. After three years of almost empty warehouses, due mainly to high world market prices and sustained demand, stocks have again built up this year.

Output in the dairy sector, the largest single farm sector, accounting for 20 per cent of the Community's total farm expenditure, is expected to rise 3.5 per cent this year, while demand within the Community is stagnating and trade frictions with the United States on export markets have led to reduced demand elsewhere. At the end of July, the European butter mountain had reached a record 770,000 tonnes, while public stocks of milk powder amount to almost one million tonnes, more than twice those of a year ago.

Surpluses are not restricted to the dairy sector. This year's harvest is expected to push up wheat stocks to almost 11 million tonnes (5 million tonnes more than last year), while barley and rye stocks have increased dramatically to 812,000 and 306,000 tonnes respectively. Beef surpluses total more than 300,000 tonnes, up from 75,000 tonnes a year ago, and olive oil stocks, although smaller than last year, still exceed 122,000 tonnes.

Moreover, Commission officials fear that a record wine crop forecast for this year might lead to a crisis similar to last summer's "wine war", when French farmers destroyed low-cost imports from Italy and Spain, leading to an open political crisis between Italy and France.

European farm support mechanisms are increasingly complicated, but this does not prevent expenses from soaring and surplus stocks from getting out of control. "The way the Community settled the wine war is typical: no structural changes, merely cosmetics", one official noted. "To ease the tensions, it agreed to finance a large-scale distillation operation involving 13.8

million hectolitres of cheap wine at a total cost of more than 400 million ECU. Then it had to subsidize the storage and marketing of the alcohol to avoid the collapse of the European alcohol market."

One possible solution is simple — in theory. Production would be curbed and guaranteed prices within the Community brought in line with world market prices. Farmers would shift from surplus production to shortage crops and, in general, there would be much more competition.

New proposals by the European Commission along these lines include output ceilings and penalty taxes on excess production, increased import duties on animal feeds, reduced aid to European beef and sheep breeders and a consumers' tax on vegetable fats. Most of these proposals have been made before and rejected by European governments. This year, they have again met violent criticism by European politicians and farm lobbies.

However, commission officials hope that the budgetary crisis now faced by the Community and the consensus to curb farm spending will increase the chances of the proposals being well received at the European summit in Athens in December. But there is little faith in Brussels that European governments are willing to make the necessary concessions and come to terms with a problem that has become a threat to the survival of the Community.

Geert Linnebank

Texas thinking big

Washington

TEXAS A&M University, which, together with the University of Texas, has received much attention lately for its efforts to turn oil and gas revenues into prestige, has bagged its first Nobel Prize winner. The university announced earlier this month that Dr Norman Borlaug, who won the Nobel peace prize in 1970 for his development of high-yielding varieties of wheat that set off the "green revolution", will join the faculty next January.

University officials were unashamedly exuberant about their coup; Texas A&M president Frank Vandiver called it "a major step forward in our quest for pre-eminence", and, added, in a very faint nod to institutional humility, "it is appropriate that our first Nobel Laureate should be in agriculture".

Borlaug will hold the position of "Distinguished Professor of International Agriculture" while continuing his association with the International Maize and Wheat Improvement Center in Mexico City. His salary at Texas A&M, according to the press office there, will be equivalent to a full-time salary of \$100,000 per year.

Stephen Budiansky

US high technology

States compete with novel bait

Washington

STATE governments have become key but inconspicuous players in the promotion of high-technology industries. In an intense competition to attract high-technology investment, 27 state governors have appointed boards and commissions to upgrade local research universities, build science parks and conjure up venture capital for private or academic entrepreneurs who can turn scientific ideas into marketable products.

Many of these commissions have become permanent parts of their state government. Early this year, Minnesota established a science and technology office to advise the governor. Iowa has set up a high-technology council with a research budget of \$2 million. North Carolina recently breathed new life into its slumbering science and technology board. And the state of New York has told its science and technology council, chaired by the state commissioner of commerce, to implement an ambitious plan to build up high technology in the region.

Although they were once viewed with scepticism, science councils at state government level appear to have become essential for states that want to attract high-technology industries. Earlier this year, 27 states competed to persuade the new \$50 million Microelectronics and Computer Corporation (MCC) to make its home in their state. According to MCC chief executive Admiral Bobby Inman, the decision went to Austin, Texas, because a state board mobilized political, financial and academic leaders and put together a package of inducements MCC could not refuse.

As part of the package, the state will sink large sums in the University of Texas at Austin to build up its strength in computer science. There will be \$15 million over three years for endowed chairs and additional professorships and \$750,000 a year for 10 years in grants for computer science graduate students. The state is offering MCC a new building on university-owned land at a minimal rent, low fixed-interest home loans for MCC staff and a job-finding service for their spouses.

Texas is only an inch ahead of other states working aggressively to increase their stake in high-technology. New York, which first set up a science and technology council in the 1960s, is spending heavily to implement a high-technology master plan, devised by the Battelle Laboratories (Columbus), that will boost the state's existing strengths in electronics, information science, medicine and biological products.

Under one programme, the state is supporting university and non-profit laboratories whose work can be rapidly commercialized. The State University of New York (SUNY) at Stony Brook, for example, is receiving money to find industrial

collaborators for its work on the production of monoclonal antibodies. Meanwhile, a state-funded corporation for innovation development gives start-up capital to small high-technology ventures. A typical recent investment was \$100,000 for Laboratory Microsystems, a Troy company that applies microcomputers to laboratory instruments.

At the centre of New York's plan is the designation of "centres of advanced technology" to nurture links between universities and companies. Advised by the National Academy of Science, the state has already spent nearly \$2 million on four centres at local universities: Cornell (biotechnology), Rochester (optics), SUNY (medical diagnosis) and the Polytechnic Institute of New York (telecommunications).

In Massachusetts, where new high-technology industries have helped to arrest two decades of decline in conventional industries, a state technology park corporation is raising funds for a network of facilities that can be used by universities and private industry to train students and employees. The first facility, a \$40 million microelectronics centre, will receive half its money from the state and half from a consortium of private companies and universities.

Like New York, Massachusetts has established a technology development corporation which tracks down venture capital for new companies or invests its own money in young companies. Last year, the corporation invested \$150,000 in Aspen Technology, a computer software company formed by members of the faculty at the Massachusetts Institute of Technology.

North Carolina, runner-up in the competition for MCC, has recently im-

proved coordination between its already strong research universities and high-technology companies. By setting up a microelectronics centre and a biotechnology centre, the state plans to harness existing research resources so they are better able to compete for federal grants and private investment. At present, the biotechnology centre is raising industrial money for a research and teaching consortium in plant molecular biology. It is also coordinating a three-university proposal for National Science Foundation funds to conduct research on monoclonal lymphocyte technology.

The National Governors Association, which has set up a task force on technological innovation, has counted 13 states with active plans to develop research parks or high technology zones modelled on North Carolina's Research Triangle or the Stanford Industrial Park in California. Six states have set up corporations to raise venture capital for high technology firms. Georgia, New York and Pennsylvania have created "research incubators" — university-linked facilities which offer inexpensive accommodation and technical services to a small number of start-up businesses.

The surge of competition has not always helped state governments. The Office of Technology Assessment says that in their struggle to woo investors, some states have offered to give new factories up to ten years relief from property taxes.

Lately, however, there have been signs that this kind of competitive bidding is falling from favour. A forthcoming report by the National Governors Association points out that no state can capture more than a small share of high technology firms. Future efforts, it says, should concentrate on supporting local research institutions and nudging existing industries in the direction of new technologies. **Peter David**

BA makes overtures to industry

Brighton

ALLYING itself to the fashionable idea that more should be done to strengthen links between academic scientists and industry, the British Association for the Advancement of Science (BA) has taken steps to involve more industrial companies in its activities. A Science and Industry Committee has been established, under the chairmanship of president for this year, Sir Alistair Pilkington, a prominent industrial scientist. There is also a new class of corporate membership, which has so far attracted 22 companies, including several banks.

To forge links with industry, Mr Bernard Dyer has joined the association's full-time staff from Imperial Chemical Industries Ltd. He will try to persuade as many science-based industries as possible to join the association, each paying a minimum fee of £500.

Mr Dyer will be approaching many com-

panies directly, but he hopes that smaller concerns will come knocking at his door. Through BA, companies will stand to become aware of technological developments and how these might be applied in specific circumstances. Later it may be possible to build up a register of academic and industrial consultants to advise on the use of new technologies.

The scheme may also act as a public relations exercise for industry in general. Mr Dyer points out that many schoolchildren may develop a lasting interest in science through membership of BAYS, the association's division for young people. If links with industry permeate to their level, the image of industry may be considerably enhanced. Last but not perhaps not least, a more active role for industry may help to improve the parlous state of BA's own finances.

Tim Beardsley

Playing games with numbers

SIR — Peter Stanbury (*Nature* 7 July, p.11) presented an interesting account of the ubiquity of π in particle physics. Your accompanying comment led me to do some quick number crunching of my own. I soon discovered that, if the number of the *Nature* volume containing the article (304) is divided into the issue number (5291) and the resulting quotient is divided by the page number on which the article is printed (11), the resulting number, 1.771, is 99.9 per cent of the value $\pi^{1/2}$. Surely this is simply a coincidence, or is it?

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SIR — In "The temptations of numerology" (*Nature* 7 July, p.11) you ask "... why are the relationships never exact?" The answer is that sometimes they are and you said yourself that "the numerologists do have a few successes to their credit". Some famous examples, which perhaps led to modern physics and society, were:

(1) Kirchhoff's observation in 1857 that the ratio of the electrical units was equal to the velocity of light. It was explained by Riemann in 1858 (see Max Mason and Warren Weaver, *The Electromagnetic Field* (1929), p.x).

(2) In 1885 Balmer gave a formula that fitted the spectral lines of hydrogen. It was explained in 1913 by Niels Bohr, and better by Dirac and Pauli in 1926.

(3) Kepler's third law was numerological, and was explained by Newton.

All three of these examples are of great historical and scientific interest. They were fairly accurate and also simple, and were right. Eddington's attempts, for elementary particles, were accurate at the time, forced, and wrong. There is of course such a thing as obviously bad numerology. The worst published one that I can recall appeared, believe it or not, in *Nature!* (185, 602; 1960).

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SIR — Your correspondent Peter Stanbury (*Nature* 7 July, p.11) has applied considerable effort and ingenuity to numerical speculations about mass ratios of fundamental particles. However, like many amateurs in science, he has missed one important point: the significance of (in)accuracies. Specifically, he takes the sum of the masses of the lightest baryon octet. He then finds that the ratios of these mass sums to the proton mass are 3.14006 and 9.812 respectively, and suggests that these numbers are "very nearly π or 3.14159" and "close to π^2 or 9.86960".

Now, using the values given in the 1982

tables¹, I obtain 3.13935 ± 0.00066 and 9.81460 ± 0.00062 for these ratios; these are not as quoted by Mr Stanbury, presumably because he used older tables. However, the chief significance lies in the errors (standard deviations here). They exclude Mr Stanbury's assignments by 3.4 standard deviations for the first and no less than 89 for the second.

In general (and it is sad to have to state this in *Nature*) any experimental result has at least two parts: the measurement itself, and the error on it. If any theory purports to predict the result, we have an immediate indication of the worth of that theory. On this basis Mr Stanbury's theory is in error by some 90 standard deviations; it therefore fails.

To forestall one possible defence, I accept that Mr Stanbury's later relations (2) and (3) do pass this particular test; but two "hits" and two bad "misses" do not justify any theory. (Since his other results depend on the choice of mass units, I decline to discuss them here.) To forestall another, I am a professional physicist, and therefore perhaps one of that stuffy establishment so feared by Mr Stanbury. But surely it does not take years of training and experience to realize that (to illustrate) if a theory predicts x , and if experiment says $2x \pm 0.1x$, then that theory is wrong.

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1. *Phys. Lett.* 111B, April 1982.

Sizewell agony

SIR — The opinion page article "End the Sizewell agony" (*Nature* 4 August, p.382) contains many factual errors. The most notable of these is the one that serves as its premise. In asserting that the Sizewell inquiry is causing a delay to the construction programme of this power station, it confuses the inquiry with a proper investigation of the economics, safety and other aspects of the proposal of the Central Electricity Generating Board (CEGB).

Despite ministerial assurances to the contrary, the inquiry is taking place in advance of the CEGB having prepared a design for construction. The Nuclear Installations Inspectorate (NII) will not grant a licence for construction until 80 outstanding safety issues are resolved. This requirement, they have repeatedly maintained, is entirely independent of the inquiry, and is unlikely to be satisfied until the end of 1984 at the earliest — long after the inquiry is over. The delay in the construction programme is due solely to the CEGB's difficulty in designing a reactor which conforms to British safety standards while also meeting its cost predictions. The

first design for this reactor was rejected in 1981 (after 3½ years of design effort) by the CEGB because it was too expensive. Far from idly kicking their heels, the design teams are frantically trying to make up for lost time.

The inquiry is an absurdity for precisely the opposite reason to the one you advance. It cannot deal with many issues which are crucial to the cost and safety of the reactor until a design is produced which the CEGB intends to construct, and which has been passed by the NII. Meanwhile, electricity consumers, not taxpayers, are footing the bill — the same consumers who, according to the CEGB's own figures, have underwritten more than 25 years of uneconomic nuclear power. Despite the apparent opportunity the inquiry provides fully to investigate these questions, you may rest assured that the public is likely to have little influence on whether, or indeed when, the CEGB's programme will go ahead.

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Life's thermal history

SIR — Presumably the organisms living around abyssal thermal vents get most of their energy by catalysing reactions between reducing substances coming up the vent, and oxygen spreading down from the distant atmosphere. Baross and Deming¹ do not explicitly say whether oxygen was present in the chambers in which they grew bacteria at unusually high temperatures. If it was present, their results may have little bearing on the origins, as opposed to the potentialities, of life.

The observation is extremely interesting. But it is not as "astounding" as Walsby² suggests. There is a good deal of parochial thinking in his annotation. Our mode of living is no doubt excellent in our environment. Hot springs and similar sites on Earth's surface are too evanescent, on an evolutionary time scale, to encourage extensive biological exploitation. However, as I have often pointed out (see, for example, ref.3), if an environment exists for a few million years where there is an exploitable chemical disequilibrium, it is unreasonable to assume that organisms will not adapt so as to make use of it. The essential points are duration and disequilibrium. It therefore seems unlikely that organisms flourish further down the hydrothermal vent because it is difficult to envisage a reaction which they could use there which would not already have taken place without biological aid.

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1. Baross, J.A. & Deming, J.W. *Nature* 303, 423-426 (1983).
2. Walsby, A.E. *Nature* 303, 381 (1983).
3. Pirie, N.W. *Nature* 235, 287 (1972); 296, 796 (1982).

Will peptides make vaccines?

The prospect of basing a new generation of vaccines on synthetic peptides keeps cropping up. Despite the promise, there is a long way to go.

SINCE proteins are relatively rigid structures, or so the crystallographers would have us believe, it would be unreasonable to expect that short runs of amino acids exposed on the surface of proteins would be held in the same conformation as that preferred by the same peptide when free in solution. By the same token, it would be unreasonable to expect that antibodies raised against a free peptide would frequently recognize the protein of which the peptide was part. Reason, however, has been confounded by facts. The frequency with which antibodies against short peptides, less than twenty amino acids long, react against the parent proteins is turning out to be remarkably high (see, for example, Niman *et al.*, *Proc. natn. Acad. Sci. U.S.A.* **80**, 4949; 1983). And so effective are some of the peptides as antigens that much effort is going into the design and synthesis of peptides that will serve as vaccines, stimulating the production of antibodies against the proteins on the surface of pathogenic microorganisms.

The attractions of peptide vaccines are clear. They would do away with the need to compose vaccines from inactivated preparations or avirulent strains of pathogenic microorganisms. They would avoid the necessity of a source of biological material, the blood of infected patients for example, from which antigenic proteins are prepared as the basis of a subunit vaccine. And they would allow the biotechnological process of using genetically-manipulated bacteria or yeasts to produce the antigenic proteins of vaccines to be replaced by a process of organic synthesis of peptides. So much for the theory.

In practice, there are many problems to be solved before any specific peptide vaccine is developed, the first of which is to identify an appropriate peptide. Generally that is a case of analysing the amino acid sequence (nowadays usually deduced from a DNA sequence) of the protein in question by a combination of the methods available for predicting which segments would be on the surface of the folded molecule. One favoured combination is first to identify helical stretches and then to find which of those contain well separated hydrophobic and hydrophilic sides, an indication that one side is on the surface face of the protein. By such means, highly antigenic peptides that induce protective antibodies against the foot and mouth disease virus have been identified and are in the process

of being evaluated as vaccines.

A similar approach to the production of peptide vaccines against poliovirus was very recently reported by Emini *et al.* (*Nature* **304**, 699; 1983), whose work is complementary to a more biological approach already noted in these columns (**304**, 395; 1983). The biological approach, pursued in Britain at the National Institute for Biological Standards and Control in collaboration with the University of Leicester, is to generate mutants of the poliovirus that are no longer neutralized by an antibody that is effective against the original virus, and then to determine which changes in amino acid sequence on the surface protein of the mutants are responsible for their escape from the antibody. That has led to the identification of a target on the poliovirus VP1 protein for one neutralizing antibody (a neutralization epitope of the protein in immunological jargon). The target is confined to an eight amino acid peptide that is therefore a strong candidate as a peptide vaccine.

That peptide (as part of an eleven amino acid peptide) is one of five synthesized by Emini *et al.* and tested for their potential as vaccines. Four of the five were recognized by a poliovirus neutralizing antibody but the only one that was able to induce neutralizing antibodies when injected into rabbits was the peptide that incorporates the eight amino acid stretch incriminated biologically. That, therefore, is the only one of the five whose further potential as a peptide vaccine is worth exploring. All is not lost for the potential use of the other five peptides, say Emini *et al.*, because each of them, although incapable of inducing neutralizing antibodies in rabbits, does stimulate the immune system of the animals in such a way as to potentiate their antibody response to a subsequent injection of poliovirus. That kind of priming of the immune system by peptides may be an alternative or an adjunct to vaccination with the pathogen.

For poliovirus, as for foot and mouth disease virus, effective vaccines already exist and any peptide vaccine will have to be measured against them both in protective and commercial terms. The position is very different for the *Plasmodium* parasites that cause malaria and against which there are no vaccines, although a start has been made this year by cloning the genes for the surface proteins of two different stages of *Plasmodium*. On page 29 of this issue,

Godson *et al.* take matters a stage further and, along the way, demonstrate that malaria is an excellent candidate for a peptide vaccine. Their efforts have been directed towards the cell surface of the sporozoite stage of the malaria parasite, the stage in which the parasite passes from mosquito to vertebrate (monkey in this case). DNA sequencing of that part of the gene for the major sporozoite protein that encodes its antigenic determinant has revealed a remarkable structure, in which a 36 base pair sequence is almost exactly repeated twelve times in tandem. There is only one variation in bases that would affect the twelve-amino acid peptide encoded by the repeated sequences.

Chemical synthesis of the peptides that correspond to either one or two identical repeats was followed by very preliminary tests of their potential as a peptide vaccine. Both were shown to be recognized by monoclonal antibodies directed against the sporozoite surface antigen.

So far, so good, but there is a very long way to go to show that an effective vaccine can really be developed from the peptide (not least because the project has to be translated from monkey to man, probably an easy task, and because it has yet to be demonstrated that the sporozoite stage of the parasite is an appropriate target, which is dubious).

It is, in every case, going to be a long slog from the identification of a candidate peptide to the production of an effective vaccine and many of the projects will fall by the wayside. There will need to be a great deal of testing of different peptides presented in different ways (for example, bound to carriers, polymerized or cyclized) to maximize the immune response. It is unlikely that any peptide vaccine will be useful in the prevention of human diseases unless it is given in conjunction with an adjuvant to boost the immune response, and since there are no acceptable adjuvants, much effort will need to be expended on their development. Finally, there will be such scientifically mundane questions as shelf life and temperature stability. Most of the progress in all these areas will not make exciting reading and is unlikely to appear in *Nature*. Indeed, much of it will be safely in the hands of pharmaceutical companies and unlikely to appear anywhere. What we can look forward to from them is news of a successful peptide vaccine, with foot and mouth disease the most likely to benefit first.

Peter Newmark

Cosmology

Matters of moderate gravity

from Virginia Trimble

GRAVITATION, while by far the weakest of the four traditional forces, nevertheless nearly always dominates in systems of sufficiently large scale. As a result, there are interesting problems in which general relativity (GR) introduces only small perturbations (bending of light rays by the Sun, for instance), others in which it is the only physics that need be considered (for example generation of gravitational radiation by colliding black holes), and even a few in which it interacts as an equal partner with other parts of physics (nucleosynthesis in the early Universe, for example). GR10—the 10th International Conference on General Relativity and Gravitation—recorded progress in problems belonging to all three classes*.

In the perturbation regime, R. Hellings (Jet Propulsion Laboratory) reported new analyses of Mercury and Mars radar-ranging data that lead to remarkably tight upper limits on deviations from the predictions of GR. The three post-newtonian parameters $\beta-1$, $\gamma-1$ and α_1 (all zero in GR but not in some other theories)¹ are at most -2.9 ± 3.1 , -0.7 ± 1.7 and $0.21 \pm 0.19 \times 10^{-3}$ respectively; the rate of change of the gravitational constant, $\dot{G}/G$, is at most $0.2 \pm 0.4 \times 10^{-11} \text{ yr}^{-1}$; and J_2 , the quadrupole moment of the Sun (which affects the orbit of Mercury like deviations from newtonian gravity²) is $-1.4 \pm 1.5 \times 10^{-6}$, consistent with the requirements of GR but not with those of competing theories. No additional data of comparable quality can be expected for many years as the Mars Viking lander is now dead.

In the strong-field regime, three long-standing problems have been resolved. The positive energy conjecture has, it appears, been proven to the satisfaction of most workers in the field. That is, we can say that the total energy of a general relativistic system can never become negative, a vital condition for stability of the theory (talks by E. Witten, G. Horowitz and M. Perry, Princeton University). The uniqueness of the Kerr-Newman solution for rotating charged black holes has also finally been established. That is, the Kerr-Newman metric is the only stationary axisymmetric solution possible within GR³. And, third, the generalized second law of thermodynamics does not break down in the presence of assorted tricky manipulations of systems previously thought to violate it. That is, a generalized entropy, composed of the usual thermodynamic entropy plus a constant times the surface areas of any black holes in the system considered, is a strictly non-decreasing quantity⁴.

In contrast, three other strong-field

problems look less straightforward than before. The stability of the Kerr (rotating, uncharged) solution, long generally accepted on the basis of numerical studies⁵, and the general validity of the solution, though never fully demonstrated, have been challenged by S. Detweiler and R. Ove (Yale University), who have found what seems to be an unstable analytic mode of the scalar wave equation in a Kerr background. Many of their assumptions will require further consideration.

Second, the cosmic censorship hypothesis clearly breaks down in at least one very special case. According to D. Christadoulou (New York University), the collapse of a dust cloud starting from rest at $t=0$ can lead to cases where light rays emanating from $r=0$ are not all trapped and the singularity is thus, in principle, observable. Other speakers (for example, A. Królak, Copernicus Astronomical Center) pointed out that the case under consideration is very artificial and believe that realistic systems will always completely censor their singularities with horizons. The definitive calculations will be exceedingly difficult, as cosmological systems, at least, approach singularities through a series of ever-growing stochastic oscillations (E. Lifshitz, Moscow University).

Third, the quadrupole formula^{6,7} for emission of gravitational radiation by orbiting objects remains a major source of controversy. T. Damour (Meudon Observatory) has treated a pair of compact objects by matching a Schwarzschild solution near each to an 'external' gravitational field obtained by iterating a post-minkowskian approximation scheme that incorporates the condition of no incoming radiation. He finds agreement with the time-honoured formula, while F. Cooperstock (University of Victoria, British Columbia), treating a system of two fluid balls initially constrained by skins and struts, then released from rest, does not. There are additional firm voters on both sides; and the resolution is perhaps to be sought in detailed considerations of the fluid mechanics of real objects (C. Will, Washington University; M. Walker, Max Planck Institute, Munich), placing the problem in our third category.

The last interactive class includes problems from galaxy formation to black hole accretion discs—and several innovative methods of solution as well. The importance of getting both the relativistic cosmology and the nuclear physics right has long been appreciated by those seeking to understand synthesis of helium and deuterium in the early Universe. The agreement between observations and such

calculations within standard Friedmann cosmologies is one of the strong reasons why the simple models are so widely regarded as valid approximations. D. Matraers (University of Cape Town) and V. Canuto (Institute for Space Studies, New York) reported, however, that reasonable agreement is also possible for non-standard models with tilt and decreasing G .

Later in the history of the Universe, initially small perturbations can grow into relativistic hydrodynamic shock waves, whose proper treatment requires inclusion of conduction and dissipation in the gas as well as relativistic self-gravity (A. Anile, University of Catania, Sicily). A first attempt (H. Sato, Institute of Fundamental Physics, Kyoto) suggests that an initial spherical void forms a compressed shell at its edges, acting like an explosive source that may form a shell of galaxies along the lines proposed by Ikeuchi⁸ and Ostriker and Cowie⁹.

Once galaxies form, some apparently develop supermassive ($\sim 10^8 M_\odot$) black holes in their cores¹⁰. A star straying too close to this nucleus will be tidally disrupted and accreted. But, in the process, relativistic tidal distortion heats the stellar interior to $\geq 2 \times 10^8 \text{ K}$, resulting in explosive hydrogen and helium burning according to B. Carter and J.-P. Luminet (Meudon; ref. 11) and G. Bickness and R. Gingold (Mt Stromlo Observatory). Thus, as described by J. Wheeler (University of Texas, Austin), a massive black hole not only eats it dinner, it cooks it and cuts it up first. Correct calculations of the digestion process are critically dependent on getting the gas viscosity right (B. Muchotrzeb-Czerny, Copernicus Astronomical Center, Warsaw).

To cope with these new complex problems, there were some new (also mostly complex) methodologies. K. Thorne (California Institute of Technology) described a membrane paradigm for the treatment of black hole electrodynamic problems, in which fictitious surface densities of charge and current on the horizon replace the (unobservable) conditions inside. And sensible application of modern computational techniques can assist in two ways. First, systems with wonderful names such as SHEEP and CAMAL (discussed by I. Cohen, University of Stockholm) can manipulate a given metric algebraically to extract the Reimann and Ricci tensors and even decide whether it is equivalent to some

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*The conference was held on 5–9 July 1983 in Padova, Italy.

other specific metric. Second, numerical methods have progressed to the point where they can handle, for instance, the initial stages of relativistic collapse of a magnetized star (K. Maeda, Institute of Fundamental Physics, Kyoto). These algebraic and numerical methods are still regarded with some scepticism by the majority of relativists. L. Smarr (University of Illinois at Urbana-Champaign) spoke feel-

ingly of the need for collaboration between the small groups working with these approximation methods and the main body of relativists with their enormous skills at finding and understanding exact analytical solutions. To which, amen. □

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Population

Nations and numbers, 1983

from Robert M. May

THE United Nations Fund for Population Activities is supported by voluntary contributions from member nations, and provides information and technical aid to countries operating programmes of family planning. According to a report recently issued by the Fund (16 June 1983), the number of people in the world is expected to increase annually at a rate of about 2.0 per cent over the period 1980–1985, which represents a significant downturn from the 2.4 per cent annual rate that prevailed in 1965–1970. Gazing to the future, through glasses that in the past have usually proved rose-tinted, the Fund suggests a continuing slowing of population growth throughout the next century, with eventual stability at a global population of slightly over 10^{10} at the end of the twenty-first century; the present global population is around 4.6×10^9 .

As reviewed in previous articles (*Nature* 287, 482; 1980 and 271, 504; 1978), there are great differences — both at present and in the probable future — between population trends in the developed and the developing (or, less euphemistically, the rich and the poor) countries. According to the United Nations Fund report, smaller families and fewer births are the key to a population's levelling off. In the industrial nations, the average annual birth rate has held roughly steady at around 16 per 1,000 of population over the past decade. In the Third World, annual birth rates are now about 33 per 1,000; this is significantly down from an average of around 37 per 1,000 in the years 1970–1975, but there is a long way to go.

The report acknowledges that overall population growth rates appear to be out-running increases in food production in developing countries, and estimates that by the end of this century such countries may have in excess of 400 million more people than they can feed. The report envisages that this problem will yield to a technological solution (use of more fertilizer, more pesticide and higher-yielding strains of crops in developing countries), although I think higher levels of import from developed countries is a more plausible hope.

Four in every ten of the world's people live in China or India today. These two

populations — approximately 1,000 million in China and 700 million in India — are in a class by themselves; the next largest populations are those of the USSR and the USA, each roughly one-third that of India. Coale's (*Proc. natn. Acad. Sci. U.S.A.* 80, 1757; 1983) careful evaluation of the demographic facts for these two countries is therefore especially pertinent to any appraisal of global trends.

At the outset, Coale emphasizes that "Data on population in China and India in the past 30 years are characterized by different kinds of limitation and uncertainty". For India, where 12 censuses have been carried out since 1872, extensive compilations of data are available, but they are

Estimated average annual birth and death rates per 1,000 in China and India

Decade	China		India	
	Births	Deaths	Births	Deaths
1950s	36	18	45	26
1960s	36	13	41	19
1970s	23	7	39	17

inaccurate: ages are often grossly mis-reported, and births and deaths not always registered. In contrast, for China the record-keeping associated with pervasive government control of daily life and the precision about birth dates associated with traditional beliefs in astrology make the possibility of obtaining accurate data " tantalizingly present"; but little attention has been given to compiling these data.

These caveats having been made, Coale presents estimates of the average annual birth and death rates in the two countries, decade by decade, over the 30 years that have passed since their emergence from largely colonial status at the end of World War II. These data are summarized in the table. We see that the average annual rate of population growth in the 1950s was slightly larger in India than in China (about 19 per 1,000, or 1.9 per cent, compared with 1.8 per cent), whereas in the 1960s the reverse was true (2.3 per cent in China compared with 2.2 per cent in India). In the 1970s, however, the average annual population growth rate in China fell to about 1.6

per cent, while remaining 2.2 per cent in India.

Although the two countries are similar in that both are relatively poor and heavily agricultural (in both, 80 per cent of the people live in the country), there are pronounced differences in birth and death rates; these tend to be obscured by the similarities in overall population growth rates. Coale attributes the lower and more improved death rate in China to two main factors. First, the relatively egalitarian distribution of income in China has meant that although "per capita availability of food is only moderately more favorable, those at the bottom of the scale are less deprived in China than in India". Second, the number of physicians per person is roughly three times larger in China than in India, and this relative abundance of physicians is also better distributed (in India, the doctor/patient ratio is eight times higher in cities than in the countryside). As important, Chinese health care is supplemented by paramedical personnel or 'barefoot doctors', and by strong emphasis on preventive medicine (including clean water supplies and the fly-swatter-implemented 'campaign against flies').

The table also shows the dramatic decline in the Chinese birth rate in the 1970s, undoubtedly associated with such centralized coercion as the requirement that couples obtain a 'planned birth certificate' before the wife becomes pregnant. In India, Coale notes, "Despite official support (for 30 years) of family planning, the government of India has not been able to organize a birth control program that regularly provides adequately staffed services to most of the population". In the mid-1970s, attempts at coercive measures not too different in principle from those implemented in China led to the fall of the Ghandi government.

In summary, Coale estimates that if present trends are sustained the population of China may stabilize at about 1,200 million by the year 2020; this represents a 75 per cent increase from the 700 million people present when the birth rate began its dramatic decline in the mid-1960s. India today has demographic parameters rather similar to China in the mid-1960s, and Coale thus estimates that even if fertility in India starts to fall soon the population is unlikely to stabilize below 1,200 million. In short, the two largest populations in the world are fated to become much larger.

In conclusion, Coale emphasizes that "A lower birth rate now is desirable, but the ideal rate is not zero. There are social and political costs of excessive emphasis on the immediate achievement of very small families; the rights and sensibilities of the current population, and the disequilibrating effects of drastic changes in age composition must enter the calculation of desirable population policies". □

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Virology

Human leukaemia virus mimics cellular antigens

from Robin A. Weiss

THE human T-cell leukaemia virus (HTLV) is yielding intriguing data on unexpected relationships between viral and host antigens that have not previously been seen with animal retroviruses. Earlier this year Haynes *et al.*¹ showed that a monoclonal antibody against the p19 *gag* antigen of HTLV cross-reacts with an epithelial antigen in the human thymus. As HTLV is not an endogenous virus² one would not have expected to find a related antigen expressed in normal tissue. Although incidental cross-reactions between otherwise unrelated proteins are increasingly noted with monoclonal antibodies, the thymus antigen has the same molecular weight as the viral antigen³ and the relationship between the two antigens seems more than chance.

It can be argued that viral p19 is not, after all, a virally coded antigen, but is a cellular antigen induced in HTLV-transformed thymus-derived cells, and that antigen becomes associated with viral core protein during assembly. This seems unlikely because the monoclonal anti-p19 antibody also recognizes a precursor protein containing both p19 and p24 antigens; p24 is indisputably a *gag* gene product and it is not expressed in the normal thymus. With the sequence of the HTLV genome known⁴, it should be possible to establish by partially sequencing the p19 protein whether it really is encoded by the *gag* gene.

Now new evidence reported in this issue of *Nature* suggests the envelope glycoprotein of HTLV is also related to cellular antigens. Mann *et al.*⁵ (see p.58) from the National Cancer Institute, Uniformed Services University of the Health Sciences and Duke University School of Medicine report that cell lines producing HTLV express supernumerary HLA specificities, which are determined by the virus. Using alloantisera and a monoclonal antibody to a set of HLA class I antigens, all HTLV-transformed cell lines are found to express an antigen not always found in uninfected cells of the same host. The new HLA specificity could be a cellular antigen induced by HTLV, but more probably it is a determinant of the viral envelope antigen. In hybrids of HTLV-infected HUT102 cells and hamster cells, the new HLA specificity segregates not with HLA on chromosome 6, but with the chromosome carrying the integrated HTLV provirus⁶.

In an accompanying paper, Clarke *et al.*⁷ (see p.60) from the National Cancer Institute show that a weak but real homology of nucleotide sequences exists between DNA clones of the *env* gene of HTLV and class I HLA. This finding makes the an-

tigenic cross-reactivity much more significant. The homologous sequences are in the coding region for the extracellular portion of these membrane antigens. It will be interesting to compare the full nucleotide sequences of HTLV *env* and the homologous region of *HLA-B*.

It is not yet clear what the biological significance might be for a viral membrane antigen to mimic a histocompatibility antigen. Mann *et al.*⁵ suggest that it might render the leukaemia patients tolerant to HTLV membrane antigens. However, our studies with HTLV pseudotypes indicate that all patients with adult T-cell leukaemia make neutralizing antibodies specific to HTLV envelope glycoprotein⁸ as well as

antibodies to viral core proteins. It will be interesting to investigate whether individuals with the HLA haplotype to which the viral antigen is related are less or more susceptible to HTLV infection and HTLV-associated malignancy.

Yet another cellular antigen associated with the HTLV envelope is the T-cell growth factor receptor recognized by the tac-1 monoclonal antibody⁹. In this case, however, it is clear that the antigen is cellular in origin and is selectively taken up from the cell membrane during budding of the virions¹⁰. □

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Stereochemistry

Handedness from symmetry and a stereochemical *coupe du roi*

from Stephen Mason

STIMULATED by an after-dinner diversion known as *la coupe du roi*, Anet *et al.*¹ have contrived a stereochemical feat that at first sight challenges a general principle originally enunciated by Louis Pasteur.

Pasteur² was the first to identify the class of handed or enantiomorphous molecules, which are characterized by structures that are not superposable by translation and rotation alone upon the corresponding mirror-image form. Such structures were termed 'dissymmetric' by Pasteur² and, subsequently, 'chiral' by Kelvin³ from the

familiar analogy of the morphological relationship between the left and the right hand.

Fischer⁴ introduced a convention to distinguish between the two mirror-image molecular forms, one being termed the D-isomer (*dextro*) and the other the L-enantiomer (*laevo*). Although still used for the α -amino acids and the sugars, the Fischer convention accumulated ambiguities of nomenclature and was replaced. In the generally adopted system of Cahn, Ingold and Prelog⁵, based on the relative mass of the atoms bonded to an asymmetric carbon atom or other chiral centre, the absolute structure of a given chiral molecule is unambiguously specified as that of the *R* configuration (*rectus*) or the *S* configuration (*sinister*).

Pasteur² maintained that, in the laboratory, the chemist without dissymmetric agents at his disposal is able to synthesize only an equimolecular mixture of *R* and *S* isomers, a racemic mixture, or an internally compensated single product, with bonded *R* and *S* moieties, the so-called *meso*-substances. Similarly, the chemical degrada-

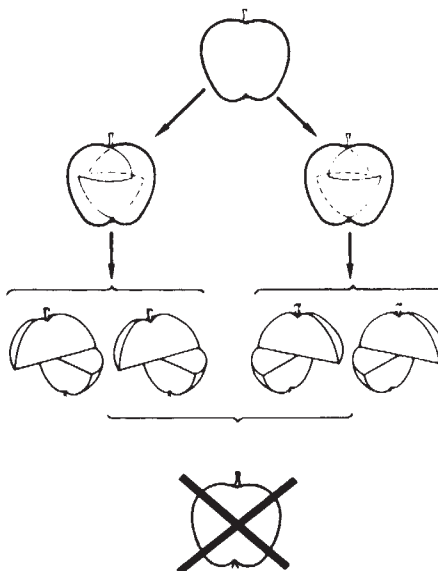


Fig. 1 The apple subject to *la coupe du roi*. Each apple yields a pair of homochiral segments, the two being identical in the ideal case of the sphere. The particular handedness of the two segments depends on the pair of opposite equatorial quadrants chosen for the horizontal cuts. A left-handed segment, given by one of the choices, and a right-handed segment, provided by the other, cannot be reassembled to form an apple.

tion of a large achiral molecule would be expected to afford, at most, a racemic mixture of chiral fragments. Schematically, the reductive cleavage of achiral *meso*-2,3-dimethyltartaric acid yields an equimolecular mixture of *R*- and *S*-lactic acid.

Anet and Miura at the University of California, Los Angeles, and Siegel and Mislow at Princeton University have now shown¹ however that the fission of an achiral dimer to racemic or achiral monomeric units is not general. If the achiral dimer is cyclic, a double cleavage is required to generate the two constituent units, and these may be homochiral, with an identical absolute stereochemical handedness.

The after-dinner diversion that stimulated that study has been practised at conferences on stereochemistry for many years, and consists in the cleavage of a sphere, usually represented by an apple, into homochiral halves. The apple, held by its stem (see Fig. 1) is cut halfway through from the north pole at the top and from the south pole at the bottom, the planes of the two semi-cleavages being orientated at right angles to one another. Two horizontal quarter-cuts, in opposite equatorial quadrants, give two half apples, each representing two quarter spheres joined at the equatorial quadrant which remains uncut (see Fig. 1). The infinite symmetry of the sphere is reduced thereby to the 2-fold rotational symmetry of each moiety. Lacking rotation-reflection symmetry elements, each half apple is chiral and, moreover, the two halves are homochiral, with the same absolute morphological handedness (see Fig. 1). The third cleavage operation, the two horizontal quarter cuts, provides a choice between the one or the other pair of opposed equatorial quadrants. The first choice generates two left-handed halves and the second gives two which are both right-handed. A left-handed and a right-handed half prove to be sterically incompatible, and the original apple shape is restored only by the union of two left-handed or of two right-handed halves (see Fig. 1).

The homochirality of the two halves produced by *la coupe du roi*, as the apple-cleavage outlined above is known in France, was discovered in 1937 by Alain Horeau at the Collège de France, Paris (see ref. 1). A chemical analogue Horeau suggests, would be the reductive cleavage of the achiral diether **1** (see Fig. 2) which contains two *meso*-bridges, each made up from a *R* and a *S* chiral centre and so internally compensated. A stereospecific chiral catalyst producing the reductive fission of each bond from an oxygen atom to the adjacent *R*-carbon atom would then yield¹ two molecules of *S*-*o*-ethyl- α -methylbenzyl alcohol, **2**. The thought-experiment of Horeau does not violate Pasteur's principle, since a dissymmetric agent is involved, the chiral stereospecific catalyst.

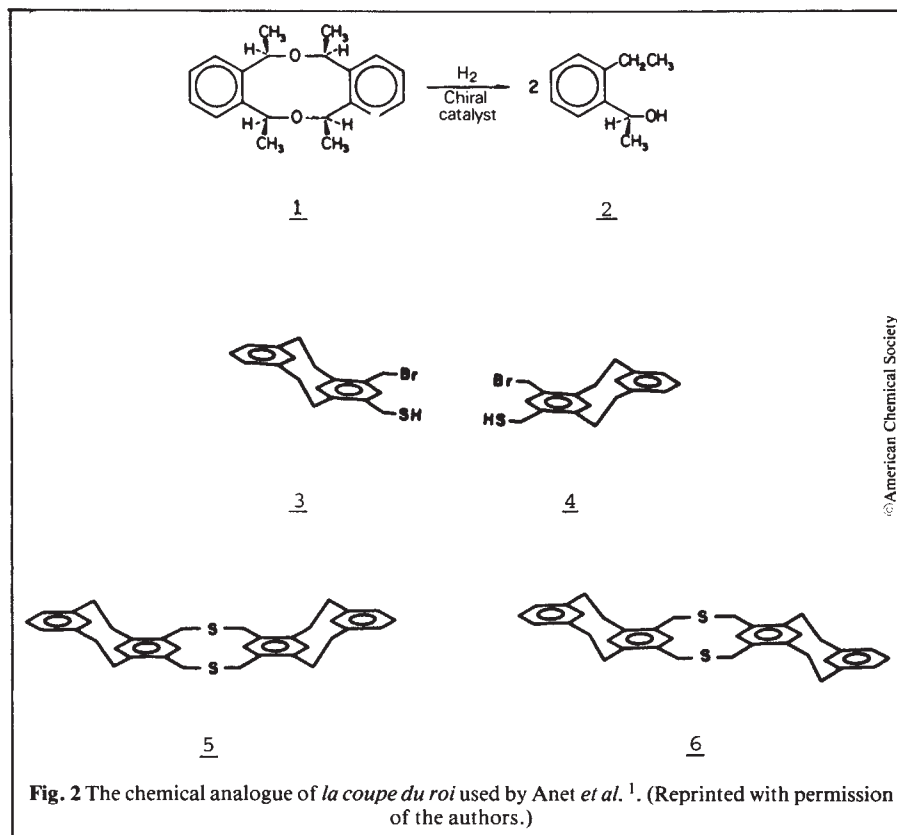


Fig. 2 The chemical analogue of *la coupe du roi* used by Anet *et al.*¹. (Reprinted with permission of the authors.)

The problem confronting the corresponding laboratory experiment, the development of the appropriate chiral catalyst, is avoided in the study of Anet, Mislow and co-workers¹ who turn round the proposed chemical degradation into a practicable analogous synthesis. The preparation of bifunctional monomers which self-couple to form cyclic dimers is readily feasible. If the monomer is chiral, it is possible to obtain an achiral cyclic dimer not only from the self-condensation of a heterochiral monomer pair, where the two molecules have non-superposable mirror-image structural forms, but also by the corresponding union of a homochiral monomer pair, where the two molecules are identical in all respects. The cyclic dimers formed in the two condensations are not identical however, and they constitute a geometrically isomeric pair, differing in structural symmetry.

The chiral monomers selected by Anet *et al.*¹ are the bifunctional metacyclophanes, **3** and **4**, which, as a racemic mixture, couple to form approximately equal quantities of two cyclic dimers, one with the *cis* structure, **5**, and the other with the *trans* stereochemistry, **6**. The individual chiral monomers, the *S* isomer, **3**, and the *R* enantiomer, **4**, are obtained from carboxylic acid precursors, which are separated from the corresponding racemic mixture by recrystallization of the diastereomeric salts formed with the chiral alkaloid, quinine. The self-condensation of two molecules of the *S* isomer, **3**, or, equally, the corresponding coupling of two molecules of the *R* enantiomer, **4**, is found

to yield only the *cis* cyclic dimer, **5**. Thus, in the racemic mixture of **3** and **4**, the heterochiral combination of one molecule of **3** with one molecule of its enantiomer, **4**, gives the *trans*-cyclic dimer, **6**, and this condensation proceeds with approximately the same probability as the corresponding coupling of two molecules of **3**, or of two molecules of **4**, to produce the *cis* isomer of the dimer, **5**.

Both dimers are achiral. The *cis* isomer, **5**, is bisected by two mutually perpendicular mirror planes, which intersect along the direction of the 2-fold rotation axis (C_{2v}), while the *trans* isomer, **6**, has an inversion centre of symmetry and is bisected by a mirror plane perpendicular to the 2-fold rotation axis (C_{2h}).

In principle, the addition of the elements of hydrogen bromide to the *cis* dimer, **5**, with the appropriate chiral catalyst, would yield homochiral monomers, either two molecules of **3**, or two molecules of **4**, depending upon the particular dissymmetric agent used. The corresponding addition of hydrogen bromide to the *trans* dimer, **6**, with or without a chiral or an achiral catalyst, would generate a racemic mixture of the monomers, **3** and **4**. □

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Animal behaviour

Foraging models and territory size

from Paul H. Harvey and Georgina M. Mace

NATURAL selection can be treated as an optimization process because it tends to maximize darwinian fitness or the reproductive success of individuals. However, applications of optimization theory to evolutionary biology and ethology do not (and cannot^{1,2}) test the general hypothesis that nature optimizes, rather they have examined specific hypotheses about the ways optimization can be achieved within certain biological constraints³⁻⁵. A recent paper by Schoener⁶, which synthesizes recent models for optimal feeding-territory size, indicates how optimality theory can be usefully applied to a field where models have usually been insufficiently precise despite detailed quantitative analyses of an extensive data base.

In a seminal paper, McNab⁷ described the empirical relationship between territory size, diet and body size among small mammal species. He noted that species living on more sparsely distributed food supplies had particularly large territories, and that the exponent relating territory size to body size within dietetic groups was similar to that relating basal metabolic needs to body size. He suggested, therefore, that territory size was determined by energetic needs. With the implicit assumption that home-ranges or territories measured in the field represent foraging areas, subsequent studies have documented similar relationships in many other vertebrate groups⁸⁻¹⁴.

More recent developments have refined these comparative analyses by relating home-range or territory sizes to estimated active daily metabolic needs (instead of body size or basal metabolic requirements)

and diet^{15,16}. The expectation has generally been that the size of the foraging area should increase in direct proportion to energy needs (that is, with an exponent of one) but this has not been borne out by the analyses; in general home-range size increases more rapidly than expected. Various explanations have been proposed for this discrepancy, the most frequent being that larger animals have a narrower size range of acceptable food items¹⁰. It has not been possible to test this idea because the relation between body size and the size range of acceptable food items is not well known in any well studied group of animals.

Not only has foraging-area size been measured and analysed in some detail, but so also has the foraging behaviour of individual animals. One of the most successful applications of optimization theory in behaviour has been to feeding strategies¹⁷. As extreme alternatives¹⁸, animals might have been selected either to minimize the amount of time spent gathering a set amount of food (time minimizers) or to maximize their energy intake in the time available for feeding (energy maximizers). An example of the application of this theory is Belovsky's¹⁹ analysis of moose diets which led him to conclude that, under the circumstances of his study, the animals were more likely to be energy maximizing than time minimizing.

A number of models for optimal territory size have resulted from the application of optimal foraging theory. However, the different models have led to quite different predictions because various assumptions have been made about the op-

timality criteria, environmental variation and the relationships between them. For example, two papers published in the same issue of *American Naturalist* predict that with increasing food density, optimal feeding-territory sizes of energy maximizers should decrease²⁰ and increase²¹. The reason for such different predictions is that the models are based on different biological assumptions.

Nine different models are reviewed and expanded by Schoener⁶, and he makes the assumptions and predictions of each one quite explicit. The models are based on two different optimization criteria — time minimizing and energy maximizing — with energy maximizers constrained either by a set foraging time or by food satisfaction (an animal can only process a certain amount of food in any given time period). The effect on optimal territory size of changing various environmental variables differs with the optimization criterion used, the biological constraints and with the form of the relationship between the costs of defence and territory area (see the table).

At present only one study purports to compare the quantitative predictions of such models using real data. Pyke²² analysed feeding-territory size in the golden-winged sunbird (*Nectarinia reichenowi*) and concluded that a model which assumed daily energy costs are minimized subject to various constraints gave a surprisingly good fit to the available data²³. Alternative models based on time minimizing and energy maximizing were less suitable predictors of territorial behaviour.

It may now be possible to identify the most appropriate models for describing why territory size changes in the way it does across the species of various vertebrate orders. As we mentioned above, many of the necessary data have already been extensively analysed, and some of the qualitative relationships between feeding-territory size

Effects of environmental change on optimal territory size defined under different optimization criteria and biological constraints

Optimizing criterion Constraint Relationship between cost of defence and territory area	Time minimizing		Energy maximizing			
			Time		Processing	
			Linear or decelerating	Accelerating	Linear or decelerating	Accelerating
Environmental change:						
Increased food density alone	—	—	—	+ or —	—	+ or —
Increased intruder rate alone	+	—	—	—	0	0 or —
Increase in food density increases intruder rate	+ or —	—	—	+ or —	—	+ or —
Increase in intruder rate decreases food density	+	+	+ or —	+ or —	+	+ or —

+, Increasing; 0, no change; —, decreasing. After ref. 6.

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and changes in environmental variables (such as food density) are well known. Previously, comparative studies and optimality theory have addressed rather different kinds of ethological issue. The application of optimal foraging models to data on interspecies differences in territory size may throw light on the precise form of cross-species relationships, as well as

demonstrating the complementarity of the two approaches. □

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Atmospheric chemistry

First results from the Solar Mesosphere Explorer

from Guy Brasseur

THE first results from the Solar Mesosphere Explorer (SME), a satellite designed specially to study atmospheric ozone, are now becoming available (*Geophys. Res. Lett.* 10, 237; 1983). The results are of particular importance given the recent worries that chemical species produced by industrial and agricultural activity might affect atmospheric ozone concentrations. Ozone is present in the atmosphere only at a low concentration but plays a vital part in protecting the biosphere from the Sun's ultraviolet radiation.

Above an altitude of about 15 km, ozone is produced from the photodissociation of molecular oxygen. In the stratosphere, its destruction is facilitated by nitrogen oxide and, to a lesser extent, by chlorine oxide, whose origin is partly anthropogenic. The study of stratospheric ozone is complicated because of atmospheric dynamics (general circulation, planetary waves and so on), which introduce a great variability in the measured concentrations and mask the effect of chemical reactions. In the mesosphere, however, the chemical time constants are sufficiently short that the ozone response to modification of the chemical or photochemical parameters should be almost immediate and practically independent of the dynamical conditions of the atmosphere. Furthermore, the mechanisms by which ozone is destroyed (established by Bates and Nicolet in 1950) are dependent only on the action of the hydrogen compounds H, OH and HO₂, which are themselves formed from water vapour.

The mesosphere therefore constitutes an ideal laboratory for the study of the chemical balance of ozone. The SME satellite, conceived at the Laboratory for Atmospheric and Space Physics of the University of Colorado, was launched into a polar orbit on 6 October 1981. Since then, it has regularly reported data which are processed directly at the Boulder control centre within a few hours.

The first results of SME are of major importance. First, they clearly confirm that in the upper stratosphere and lower mesosphere, ozone concentration and temperature are inversely related, as

foreseen by the Chapman theory in 1930, and show that atmospheric temperature is the principal cause of changes in ozone density in the lower mesosphere. As the temperature decreases, the ozone density increases, and vice versa. In the vicinity of the stratopause (50 km), ozone is mainly lost through its recombination with atomic oxygen: $O + O_3 \rightarrow 2O_2$, a reaction which has a direct temperature dependence and which therefore introduces an inverse dependence between ozone density and temperature.

Observations made by the SME limb scanning ultraviolet spectrometer between 48 and 66 km altitude¹ and by the limb scanning infrared spectrometer between 50 and 90 km (ref. 2) show that the ozone concentration varies greatly in time and space and that its behaviour is closely related to atmospheric dynamics. Below 60 km, a seasonal variation appears in the mixing ratio, with the largest values during the winter period, especially at high latitude. Above 80 km however, the mixing ratio maximum is formed near the summer pole.

Measurements in the region above the mesopause (85 km) also show a dramatic daily variation in ozone concentrations, suggesting rapid fluctuations in the atomic oxygen concentration caused by vertical motions transporting odd oxygen from above.

The density of nitrogen dioxide (NO₂) as well as the temperature have been inferred from measurements provided by a visible light spectrometer. The NO₂ density³ varies from pole to pole and during the winter months exhibits considerable variability, reflecting the much larger degree of meridional movement of air in the winter stratosphere. The observations confirm that the periods of high NO₂ at high latitudes occur when air flow is from the equator to the pole; low NO₂ occurs when the flow is reversed. Oscillations in the meridional circulation are connected with sudden warmings in the spring high-latitude Northern Hemisphere.

The SME satellite also carries a spectrometer for measuring variations in the solar irradiance and a proton alarm system which can detect the precipitation of ener-

getic particles — caused by a solar proton event⁴, for example. The largest event in the current solar cycle occurred on 13 July 1982, and the notable decrease of ozone concentration it caused was measured by SME. Proton events of this kind inject large numbers of high-energy protons into the middle atmosphere, changing the concentrations of ionized hydrogen, nitrogen and oxygen, and thus the ozone destruction rate and density. SME's near-infrared spectrometer observed ozone depletion reaching 70 per cent at 65° latitude, 78 km altitude, on the morning side and 10–20 per cent on the afternoon side.

All the observations, including those of the proton event, have been satisfactorily compared with a particularly elaborate two-dimensional model which takes into account most chemical reactions related to the mesosphere and the fundamental dynamical mechanisms^{5,6}.

The enormous advance the SME satellite has made is to present an impressive series of data at different latitudes and for different seasons. Rapid processing of the primary data is enabling it to be effectively exploited. Measurements that are applied to the mesosphere will, however, have to be supplemented by programmes that study the stratosphere, where ozone chemistry is far more complicated. Stratospheric balloon measurement experiments and, in particular, the Balloon Intercomparison Campaign (BIC) sponsored by NASA, the Chemical Manufacturers Association and the Commission of the European Communities, are not limited to ozone data, but are also devoted to molecules such as HCl, HF, HNO₃, NO and NO₂ or radicals of the OH and ClO type. They remain important together with the ground-based and aircraft-borne measurements.

The BIC, conducted from the National Scientific Balloon Facility in Palestine, Texas, is intended to enable 17 scientific instruments, developed by teams from Canada, Europe, Japan and the United States, to perform almost simultaneous measurements up to 45 km altitude. Another campaign, called Global Budget of Trace Species (GLOBUS), will be held in September 1983. Its principal objective is to measure ozone and the nitrogen oxides, using a series of balloons which will be launched from the French base of Aire-sur-l'Adour. Complementary observations of stratospheric ions and aerosols will be performed, and should enable a determination of the stratospheric effects of the El Chichon volcano whose eruption in April 1982 injected a dust cloud very high into the atmosphere. □

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Meteorology

Anomalous El Niño of 1982–83

from S. G. H. Philander

AN El Niño event, during which anomalously warm surface waters cover the central and eastern tropical Pacific Ocean, occurred between May 1982 and June 1983. The event was exceptionally intense and was associated with several natural disasters which received extensive coverage in the press. Ecuador and northern Peru had catastrophic floods because of unusually heavy rainfall. (Guayaquil had nearly 3 m of precipitation above normal between October 1982 and June 1983.) The neighbouring regions to the north and south, however, experienced severe droughts. Several hurricanes devastated islands in the central tropical Pacific, a region where such phenomena are extremely rare. Marine life practically disappeared from the upper ocean in the central and eastern equatorial Pacific¹. This, together with the flooding of bird nests, caused a decimation of the bird population on Christmas Island². Discussions of the meteorological and oceanographic aspects of this El Niño attracted considerable attention at a recent meeting* and can be summarized as follows.

The 1982–83 event evolved in an unusual manner. During a typical event⁴ exceptionally warm surface waters first appear off Ecuador and northern Peru in February and March and then expand westwards over the next several months. In 1982, however, the warm water in the western Pacific gradually spread eastwards into the normally cold eastern Pacific (R. Reynolds, NOAA, Washington DC)³. Not until September did sea-surface temperatures in the eastern tropical Pacific increase sharply to values as much as 6°C above normal. These conditions persisted through June 1983, whereas a typical event would have ended in March or April.

Rainfall estimates derived from satellite measurements by O. Garcia (NOAA, Boulder)³ indicate that the eastward expansion of the unusually warm surface waters was accompanied by an eastward displacement of the atmospheric convective region which is normally near Indonesia. This contributed to the drought in the far western Pacific and the late monsoons over the Indian Ocean. During a typical El Niño the convective region moves to the neighbourhood of the dateline, but during the past year it moved much further east, into the central and eastern Pacific so that the Southern Oscillation Index, the pressure difference between Darwin and Easter Islands, fell to record low values. A. Krueger (NOAA, Washington DC)³ described how, because of the convergence

of the surface air into the convective region, the trade winds were replaced by westerly winds over a region which at first was confined to the west but which expanded eastwards concurrently with the eastward movement of the convective zone and high sea-surface temperatures. This convergence caused an intensification of the trade winds over the Atlantic Ocean and probably contributed to the drought in northeastern Brazil.

The convection not only modified the zonal circulation in the tropics, but intensified the meridional atmospheric circulation (the Hadley Cell). The rising air in the equatorial convective region of heavy rainfall descended between latitudes 10° and 25°N and 25°S so that southern Peru and Bolivia south of the Equator, and central America north of the Equator, experienced severe droughts. The unusual convective activity near the Equator, in addition to intensifying the Hadley Cell, excited disturbances that radiated polewards into the extratropics. This affected the atmospheric variability in higher latitudes so that El Niño can be implicated in the unusual weather over large parts of the United States earlier this year (J. Horel, Scripps Institute of Oceanography)³.

The increase in sea-surface temperatures in the central and eastern tropical Pacific Ocean was caused by an enormous flux of heat from the warm western tropical Pacific to the normally cool eastern tropical Pacific (A. Leetmaa, NOAA, Miami; D. Halpern, NOAA, Seattle)³. The depth of the thermocline and the sea level decreased west of the dateline but increased in the central and eastern Pacific. (Off South America the thermocline deepened by as much as 70 m and sea level rose by as much as 40 cm)⁵. Anomalous currents advected the heat eastwards⁵. Hansen's⁵ measurements with drifting buoys reveal that, within 5° latitude of the Equator, the normally westward surface flow was predominantly eastwards. (Halpern's^{3,5} measurements by means of current meters on a mooring at 110°W on the Equator show that the westward surface flow there reversed direction in August 1982.)

The zonal redistribution of heat in the equatorial Pacific caused the eastward pressure force associated with the zonal density gradients to weaken and even to vanish for a while⁶. As a result the eastward equatorial undercurrent, which is driven by this pressure force, disappeared temporarily^{3,5,6}. This is the first time that measurements have failed to reveal the presence of this normally intense jet in the equatorial Pacific.

Interactions between the ocean and atmosphere are of central importance in El

Niño events. The ocean responds primarily to the surface winds. Normally the easterly trade winds drive westward surface currents at the Equator, expose cold water to the surface in the eastern Pacific and accumulate warm water in the west. When the trade winds relax during El Niño anomalous eastward surface currents redistribute the heat zonally. The warming of the eastern tropical Pacific can therefore be attributed to the weakening of the trade winds. But the relaxation of the trade winds, in turn, is a consequence of the exceptionally high sea-surface temperatures in the east because the surface winds converge into that region where the atmosphere is heated. The symmetry of these arguments implies that unstable interactions between the ocean and atmosphere are possible and can cause the amplification of modest initial anomalies simultaneously in the ocean and atmosphere⁴. The instability is readily initiated in equatorial regions where sea-surface temperature gradients are large — off the coast of South America for example — but on infrequent occasions, such as 1982, can also be initiated in the western tropical Pacific where such gradients are small. A crucial feature of this theory is that anomalously warm surface waters must cause a local heating of the atmosphere. This requires that there be rising air over the anomaly. The large-scale atmospheric circulation, and the seasonal movements of its convergence zones, therefore modulate the instability. Initial amplification of a modest anomaly depends on favourable large-scale conditions, but once perturbations have attained a sufficiently large amplitude they can significantly alter the large-scale flow and can induce conditions favourable for their persistence. Hence the persistence of the 1982–83 event could be a consequence of its intensity.

The explosion of El Chichón deposited a large amount of volcanic dust in the upper stratosphere. There is no reason to believe that this directly affected motion in the lower troposphere or that it influenced the evolution of El Niño.

In summary, El Niño of 1982–83 was unusual in several respects: it first appeared in the western tropical Pacific and expanded eastwards; it attained an exceptionally large amplitude; and it persisted longer than the more common events. El Niño of 1982–83 is the best documented one yet. Further analysis of the data will permit a critical examination of the proposed explanations for these events. □

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*A meeting of the American Geophysical Union was held in Baltimore on 1 June 1983.

Evolutionary biology

Taxonomy by nucleotides

from Jared M. Diamond

SHOULD a molecular biologist scan the journal *Emu*, which publishes results of research on Australian birds, his/her attention would probably not be caught by a title beginning 'The relationships of the Australo-Papuan scrub robins *Drymodes* . . .'. Even if the abstract were correct in claiming these thrush-like birds to be more closely related to Australian robins (*Eopsaltria*) than to true thrushes (*Turdus*), who cares? In fact, the series of papers by Charles Sibley and Jon Ahlquist of Yale University, with this and other such seemingly innocent titles, represents the most ambitious and fundamental effort to date to revolutionize taxonomy by using the methods of molecular biology¹⁻¹⁷.

Traditional taxonomy has been based on morphological characters, occasionally behavioural ones. These have at least three basic disadvantages. First and foremost, they are generally adaptive and not just neutral markers of ancestry. Second, anatomical and behavioural characters are subject to some phenotypic variation unrelated to genetic affinities. Finally, if one attempts to overcome the unreliability of single characters by studying many characters, it is unclear how these should be combined into a single measure of genetic distance.

In the 1950s and 1960s it was realized that comparison of protein sequences or electrophoretic patterns offered potential advantages, by basing phylogenetic conclusions on primary gene products. From 1957 to 1973 Sibley pursued electrophoretic studies of egg-white proteins, eye-lens proteins, plasma proteins and haemoglobins, with the goal of unravelling bird phylogenies¹⁸⁻²⁴. Although these studies offered new insights, it became apparent that proteins share some basic difficulties with morphological and behavioural characters as taxonomic tools. For proteins, as for bones, it can be difficult to decide whether similarity reflects shared ancestry or convergent evolution, and whether dissimilarity reflects distinct

ancestry or adaptive radiation²⁵. Amino acid sequences of proteins are fixed genetically, but the rates of synthesis and degradation of each protein, hence the amount present, are under phenotypic control. Rates of evolution vary over 100-fold among different types of protein and also vary with time and among species for the same protein²⁶⁻²⁹. Even the electrophoretic pattern of all the egg-white proteins samples only a tiny fraction of a bird's genotype and does not readily yield a quantitative measure of genetic relatedness.

A more promising taxonomic tool is the use of DNA-DNA hybridization^{30,31}. Double-stranded DNA consists of two strands with complementary nucleotide sequences that can be separated, by heating, into single strands and which reassociate on cooling. If single strands from two related species are combined in conditions permitting reassociation, the mismatched base pairs due to divergence of the species reduce bonding strength so that the dissociation temperature is lower than for double-stranded DNA from the same species. With this technique it turns out that a lowering of the dissociation temperature by 1°C corresponds approximately to a 1 per cent difference between the nucleotide sequences of the two species under comparison. Thus, the difference in temperature for 50 per cent dissociation (ΔT_{50H}) of conspecific DNA and hetero-specific DNA provides an average measure of similarity for the whole genome. As a rough rule of thumb for birds, different species of the same genus yield a ΔT_{50H} value of up to around 4°C; members of different genera but of the same family, ~4–11°C; members of different families but the same order, ~11–20°C; and members of the same class but distantly related orders, about 25°C (refs 4,6,13,15,16).

The world's 9,000 species of birds offer especially suitable material for re-examining the taxonomy of an entire class by this method. No group of animals has

been more intensively studied than birds. Yet taxonomic problems arising from convergent evolution and adaptive radiation are extreme for birds, so that taxonomists disagree widely about the relations between the groups of songbirds, and about the group affinities of many individual species.

Over the past nine years Sibley and Ahlquist have extracted and purified DNA from erythrocytes of about 1,000 bird species, including members of all recognized orders and 163 of the 171 families recognized in some traditional classifications. The table and figure illustrate the results for the ratites (ostrich-like birds) and tinamous. From such matrices of genetic distance among species emerge numerous important conclusions of which the following are samples.

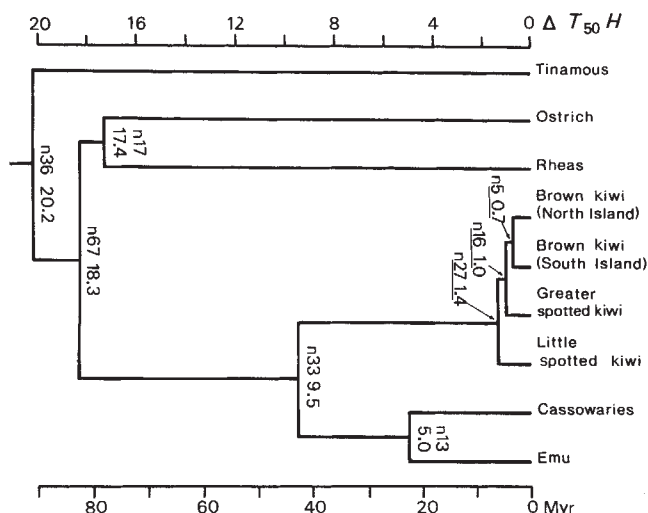
Scattered around the world are eight species or genera of songbirds adapted to creeping up vertical tree trunks or rocks and extracting insects from crevices. These are *Sitta* (Eurasian and North American nuthatches), *Certhia* (Eurasian and North American tree creepers), *Tichodroma* (Eurasian wall creeper), *Daphoenositta* (Australian 'nuthatches'), *Climacteris* (Australian 'creepers'), *Salpornis* (an Afro-Asian 'creeper'), *Hypositta* (a Madagascar 'nuthatch') and *Rhabdornis* (Philippine 'creepers'). They show characteristics such as a slender bill for probing and strong feet with long claws for gripping, and some have stiffened tails for support on vertical surfaces. Are these morphological similarities due to shared ancestry or to convergent evolution under similar selective pressures? DNA hybridization studies support the latter conclusion: ΔT_{50H} for *Daphoenositta* is 12–14°C vis à vis *Sitta*, *Certhia* and *Climacteris*, but only 7–8°C vis à vis some ecologically and morphologically very dissimilar Australian birds, which are evidently *Daphoenositta*'s closest relatives¹⁰.

When European zoologists first explored Australia, they found animals resembling European mice, cats, moles and wolves. However, these diverse mammals were not placental and shared a pouch and skeletal and dental features, demonstrating them to be marsupials related to each other and convergent on their European look-alikes. The marsupials now furnish the classic textbook example of adaptive radia-

Lowering of dissociation temperature of hybrid DNA, as a measure of genetic distance

	Cassowary (Australia, New Guinea)	Emu (Australia)	Brown kiwi (New Zealand)	Rhea (South America)	Ostrich (Africa)	Tinamou (South and Central America)
Cassowary	—	—	—	—	—	—
Emu	5.0 ± 0.05(13)	—	—	—	—	—
Brown kiwi	9.6 ± 0.1(18)	9.4 ± 0.1(15)	—	—	—	—
Rhea	18.2 ± 0.2(8)	18.4 ± 0.1(13)	17.9 ± 0.1(11)	—	—	—
Ostrich	18.3 ± 0.1(11)	18.6 ± 0.1(12)	18.4 ± 0.1(13)	17.4 ± 0.1(17)	—	—
Tinamou	20.3 ± 0.1(7)	19.9 ± 0.2(6)	20.1 ± 0.1(7)	20.2 ± 0.2(12)	20.4 ± 0.3(6)	—

Average value ± 1 s.e.m. of ΔT_{50H} values (°C) for DNA hybrids using all pair-wise comparisons of the five main groups of ratites (flightless ostrich-like birds) and the tinamous. Modern geographical ranges of each group are given above. Number of DNA hybrids measured is given in parentheses. ΔT_{50H} values increase with base-pair mismatch and genetic divergence between the two species. For example, ostrich and rhea are equidistant from cassowary, while emu is much closer to cassowary. The data were kindly provided by Sibley and Ahlquist and update their data in ref.2.



Cladogram of the ratites and tinamous, constructed by Sibley and Ahlquist from the DNA $\Delta T_{50}H$ values of Table 1 (left-hand ordinate). The right-hand ordinate is the estimated time of divergence in millions of years (Myr), calculated on the assumption that $\Delta T_{50}H = 1^\circ\text{C}$ corresponds to 4.5 Myr. Numbers at branching points give the average $\Delta T_{50}H$ value ($^\circ\text{C}$) for hybrids involving the two branches, while n is the number of hybrids averaged. The oldest divergence is that of the tinamou from the ratites. The reconstructed sequence of divergence among the ratites themselves is in excellent agreement with considerations based on the breakup of Gondwanaland and subsequent continental drift. The first rift was between Australia and the other southern continents (node at $\Delta T_{50}H = 18.3^\circ\text{C}$), followed closely by the opening of the Atlantic between Africa and South America (17.4°C). The Tasman Sea between Australia and New Zealand opened around 80 Myr ago. The $\Delta T_{50}H$ value of 9.5 for the node between the kiwis and emu-cassowaries indicates that this divergence occurred much later, around 40–45 Myr ago. Thus, the ancestor of the kiwis must have reached New Zealand from Australia via intervening island arcs or emergent land along the rises that bear Norfolk and Lord Howe Islands today. The three species of kiwis have diverged within New Zealand in the last 10 Myr (1.0 – 1.4°C), and the two subspecies of Brown Kiwi even more recently (0.7°C).

tion. The zoologists also found some songbirds resembling European nuthatches and creepers (as just mentioned), warblers, thrushes, robins, chats, wrens and flycatchers. These bird look-alikes shared no distinct feature like a marsupium. Thus, they were often placed in the corresponding European bird families. The DNA data make clear, however, that most Australian songbirds are descended from a single adaptive radiation paralleling that of the marsupials^{8,10–12,15,16}. This radiation produced Australia's 'nuthatches', 'warblers', 'flycatchers' and other look-alikes; its distinct lyrebirds, bowerbirds, birds of paradise and honeyeaters; and several groups of birds that are now found worldwide (crows) or throughout the Old World (orioles and cuckoo-shrikes) and that were not previously recognized as having been of Australian origin.

Existing classifications of songbirds prove in the light of DNA data to abound with misplaced taxa and artificial (polyphyletic) groupings, and to conceal other adaptive radiations. The conventionally recognized family *Muscicapidae* (Old World flycatchers) turns out to contain members of both major branches and four of the six superfamilies of songbirds defined by the DNA studies^{1,15}. The New World tanagers (Thraupini) constitute a previously unrecognized adaptive radiation whose members had been scattered among five 'families' including finches and honeycreepers¹⁷.

Since the widespread acceptance of continental drift by geologists, biogeographers have accepted that the biotas of Australia, South America, New Zealand, Madagascar and other continental fragments are a mixture of two elements: some whose ancestors were already present before drift isolated the continent or island and others whose ancestors arrived subsequently by overwater dispersal. An extreme school of vicariance biogeography minimizes the role of overwater dispersal and implicitly assumes most components of the biota to share a divergence time equal to that elapsed since the rifting. This view ignores the obvious fact that biotas contain elements of very different degrees of endemism. The DNA data make this difficulty quantitative^{8,10,15,16}. The $\Delta T_{50}H$ values by which Australian birds differ from their nearest Eurasian or New World relatives differ enormously: from 18°C for Australia's endemic suborder of emus and cassowaries (vis à vis African ostriches and South American rheas) to less than 1°C for the Australian endemic crow species *Corvus mellori* (vis à vis Eurasian and North American crows). The ages of divergence must differ proportionately. The flightless emus and cassowaries are probably descended from ancestors already present in Australia before the break-up of Gondwanaland; most endemic Australian songbirds, from an overwater colonist arriving around 65 million years

ago; the crow, from an Asian crow arriving within the past few million years, that Asian crow in turn being derived from an Australian songbird that colonized Asia around 35 million years ago; and the newly self-established Australian population of the Sarus Crane (*Grus antigone*), from immigrants arriving from south-east Asia in the past few decades.

When biochemical techniques were introduced to taxonomy, they were greeted with exaggerated hopes by some and with hasty dismissal by others. It is now clear that even the DNA hybridization method requires careful attention to technical detail, like any taxonomic tool. It is also clear that the technique has been adequately validated on taxa of undisputed affinities and cannot be dismissed.

When the calibration of $\Delta T_{50}H$ to absolute dates becomes more secure, the work of Sibley and Ahlquist may for the first time provide taxonomists with a cladogram that has dated branching points for all extant families of an entire vertebrate class.

Now that phylogenies can be deduced completely independently, evolutionary interpretations of morphology will rest on a secure base. Sibley's and Ahlquist's discoveries of the radiations of Australian songbirds and New World tanagers illustrate the possible pay-offs. Thus, DNA hybridization may permit a new flowering of traditional morphology. □

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An oscillating trend in Phanerozoic non-skeletal carbonate mineralogy

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Textural and compositional data on ancient ooids and cements suggest the existence of an oscillating Phanerozoic temporal trend in non-skeletal carbonate mineralogy. The trend shows good correlation with eustatic-climatic oscillations proposed by others. The most likely mechanism appears to be plate-tectonically influenced oscillations in P_{CO_2} , the vapour pressure of CO_2 , rather than change in oceanic Mg/Ca ratio.

FOR several decades, advances have been made in the understanding of ancient carbonate rocks through study of modern carbonates and their environments of deposition and post-depositional alteration (diagenesis). Recently, increased attention has been given to the probability that significant differences exist between modern carbonates and some ancient carbonate systems. In 1975, based on limited observation of textural preservation or disruption in ooids, I suggested¹ a shift in the early Cenozoic from conditions favouring precipitation of ooids (and, by implication, non-skeletal carbonates in general) as calcite, to the aragonite-facilitating conditions which still prevail. More recently, Mackenzie and Pigott² (based in part on other work^{3,4}) have suggested that the change occurred in about mid-Carboniferous time. My recent investigations of ooids and cements, discussed here, indicate that both suggestions may be correct. The implication of the data is that, rather than a single unidirectional shift, there is an oscillating global trend in non-skeletal carbonate mineralogy, at least since late Precambrian time (Fig. 1).

The interpretation of mineralogical indicators in ancient ooids and cements presented here suggests several first-order cycles in original non-skeletal carbonate mineralogy. Those cycles, which generally follow the eustatic-climatic cycles proposed by others^{2,5-8} (Fig. 1), include two 'aragonite-inhibiting' episodes (Cambrian to about late Mississippian, and late Triassic or early Jurassic to early or mid-Cenozoic). Alternating with those episodes are three 'aragonite-facilitating' episodes (late Precambrian to at least early Cambrian, late Mississippian to late Triassic or early Jurassic, and early or mid-Cenozoic to the present).

A major difference between the present study and that of Mackenzie and Pigott is in their interpretation^{2,3} of original aragonite mineralogy for most Jurassic and Cretaceous oolites they studied (9 out of 14, from University of Texas collection). My results are strikingly different. Of 32 Jurassic and 23 Cretaceous units studied (from North and South America, Europe, Africa and Asia), including the University of Texas thin sections, none showed reliable evidence (criteria (1)–(4), see below) for original aragonite, although such textures were seen in a large percentage of Pennsylvanian to Triassic units (Fig. 2). However, some units, particularly in the Jurassic, Cretaceous and Mississippian, showed selective, usually partial dissolution of ooid cortices.

Recognition of ancient aragonites

From the literature, it is evident that calcite constituents of virtually any textural state, from mouldic to fine-textured (presumed to be pseudomorphic), have been inferred by some authors to have been originally aragonitic. My studies on diagenesis of unquestionably aragonitic (mainly skeletal) con-

stituents, both published^{1,9,10} and unpublished, indicate that some of those textural states are not valid indicators of aragonite. Presumed criteria for recognition of ancient aragonites can be ranked in a hierarchy of decreasing reliability, as follows: Criterion (1): preservation as aragonite. This is obviously the most reliable state, and one found in even quite old rocks¹¹⁻¹⁴. Criterion (2): relatively coarse calcite mosaic, generally irregularly cross-cutting original structure (as shown by 'organic' relics or fluid inclusions) and containing oriented relics of the original aragonite as solid inclusions^{1,9,10}. This state is scarcely less reliable than criterion (1). Criterion (3): calcite mosaic as for criterion (2), without aragonite relics, but with a distinctly higher Sr content¹⁵ than clearly calcitic constituents. The reliability of criteria (3)–(5) can be strengthened appreci-

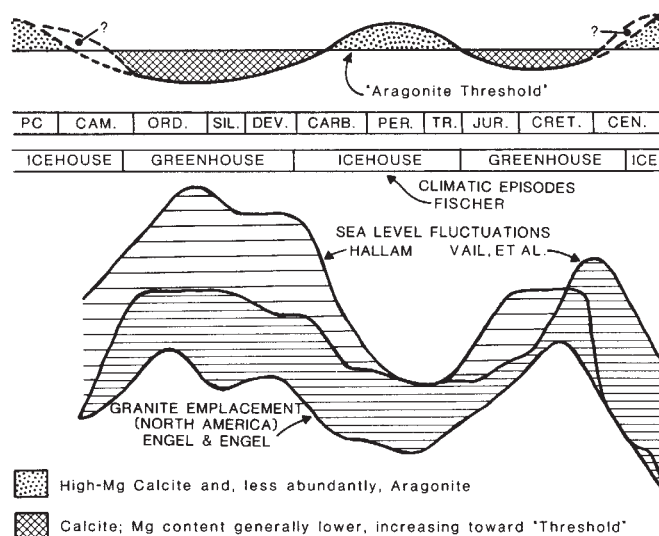


Fig. 1 Inferred first-order cycles in non-skeletal carbonate mineralogy, involving oscillations above and below an apparent 'aragonite threshold'. Those cycles are compared with the sea-level, granite emplacement curves and climatic episodes as given by Fischer⁶. The late Precambrian–Cambrian aragonite-facilitating episode is suggested by the eustatic and climatic data⁵⁻⁸ and supported by observations on botryoidal early Cambrian cements⁴⁴ (criterion (4), with ray crystals) and an early Cambrian oolite (Caribou Mountains, British Columbia, Canada) and on late Precambrian apparent aragonites (M. E. Tucker, personal communications). Similar earlier Precambrian episodes are implied by some recent work⁴⁵. Exact timing of the Cambrian and Cenozoic shifts is uncertain.

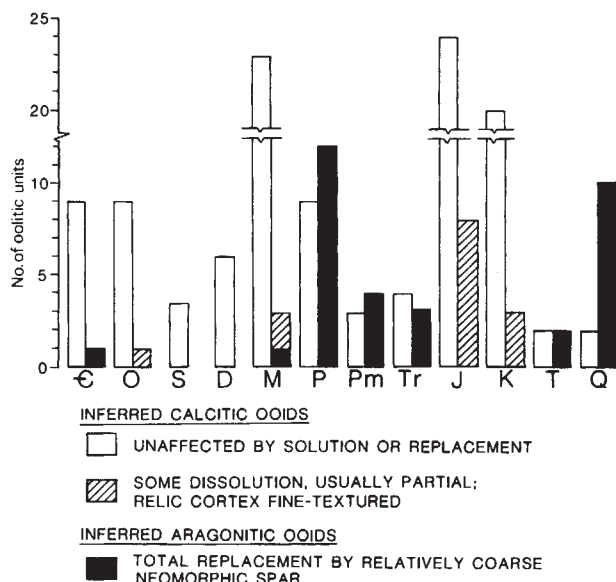


Fig. 2 Major preservation states of Phanerozoic, marine ooids. All samples are of presently calcitic ooids, except that the Quaternary total includes oolites with ooids still aragonite or partly calcitized. The oolite in Fig. 3 is not included (see text).

ably, for cements, if the external morphology shows the large square-ended ray crystals (raggioni¹⁶) diagnostic for aragonite. Criterion (4): calcite mosaic as for (3) above, but with Sr content not unusually high or not analysed. Incomplete coarse calcite replacement of otherwise fine-textured calcitic constituents, transecting depositional textures, requires assumption of pseudomorphing (criterion (6)) of the remaining fine-textured part. Criterion (5): moulds, or cement-filled moulds. Without some additional qualification, this is not a totally reliable indicator of original aragonite. Criterion (6): retention of fine structure, presumably as the result of a pseudomorphic calcitization. The last two criteria are particularly suspect and warrant further consideration.

Pseudomorphism and original mineralogy

Ooids in an Ordovician oolite (TC 630 00k), interpreted^{2,3} as at least partly aragonitic, exhibit coarse replacement calcite characteristically showing only partial irregular replacement of otherwise fine-textured, concentrically-laminated calcite layers (Fig. 3). Although bimineralic ooids are known from a variety of Holocene marine and lacustrine settings, they are always composed of discrete, concentric, monomineralic increments^{17,18}. To interpret the partially replaced layers in the Ordovician ooids as originally aragonitic requires the inference of pseudomorphic calcitization of the fine-textured remnant portions. Bimineralic oolitic units, whether as two-phase ooids (common in the Pennsylvanian of south-east Kansas) or as mixed deposits of both calcitic ooids and aragonitic ooids (Figs 4, 5), are clearly recognizable by the dichotomous diagenetic behaviour of those two types of ooids or ooid layers.

Pseudomorphic calcitization of aragonite has been most commonly invoked to account for preservation of fine texture in ancient ooids and cements. Although rarely stated explicitly, such suggestions are implicitly based on a uniformitarian assumption of original mineralogical equivalence between those constituents and modern aragonitic ooids or cements. It is the premise of this and earlier papers^{1,2} that such an interpretation of mineralogical equivalence is unwarranted for many non-skeletal carbonates in much of the geological record (the same arguments apply to skeletons¹⁰). Nevertheless, such *a priori* assumptions have led many authors to infer an intervening

'texturally-retentive' calcitization—that inferred change has been characterized as a 'very fine scale' process of 'inversion'. Bathurst¹⁹ has noted, however, that the portion of the concept of 'inversion' applicable to aragonite diagenesis is calcitization by solution–re-deposition. That form of alteration differs from wholesale solution removal and later deposition of void-fill calcite fundamentally only in scale and relative timing⁹. The term 'inversion' has unfortunate implications of solid-state alteration and should be avoided; other suitable terms (calcitization, neomorphism) exist.

The replacement textures which occur in partially calcitized aragonite constituents are particularly instructive in this discussion. In such examples, one can see that dramatic textural disruption takes place at the diagenetic front^{1,9,20}. That disruption shows no systematic decrease in examples with thinner fronts, and is still pronounced even across fronts possibly as thin as 150 Å (ref. 20). Note that, where original aragonite mineralogy of presently calcitic constituents (including skeletons, ooids, and even cements) can be confidently substantiated by properties (for example, aragonite relics, Sr content, taxonomic affinities) other than the overall texture, pseudomorphing does not occur. Neither the fine crystal morphology nor the regular morphological or optical orientation of the precursor aragonite is preserved.

Mouldic or partial mouldic preservation has been interpreted^{3,21} as indicative of original aragonite mineralogy in ooids. My examination of Jurassic and Mississippian oolitic units indicates that an uncritical acceptance of 'mould = aragonite' is potentially quite misleading. In various oolitic units, such as the Great Oolite (Bathonian, Bath, UK), the Ste Genevieve and Starrs Cave Oolites (Mississippian, Illinois Basin), there is frequently selective dissolution of ooid cortices, leaving vacant or cement-filled oomoulds, commonly with dropped nuclei. Partial oomoulds with residual fine-textured segments of cortical layers are most common. Those cortical remnants commonly show a pseudouniaxial figure, indicative of the regular radial optic orientation. Attribution of the partial dissolution to original aragonite mineralogy requires invocation of pseudomorphic calcitization of the cortical remnants. The lack of support for such an assumption was discussed earlier. An additional factor argues against originally aragonitic ooids in the Great Oolite. Coexisting molluscan remains are now micrite envelopes filled with a drusy, clear calcite spar, identical both in texture and cathodoluminescence to intergranular cement. Those shells were dissolved and the moulds were filled relatively early in diagenesis. In contrast, the oomoulds and partial oomoulds rarely contain cement and may, at least in part, be a very late, possibly near surface phenomenon. The significant point is that the shared tendency for dissolution of ooids and molluscan shells cannot be taken as an indication of mineralogical equivalence. Some texturally altered Jurassic ooids illustrated by Sorby (Kelloway Rock, ref. 22, plate X, Fig. 2; Text—Fig. 7) which contain centripetally arranged, bladed secondary calcite have been regarded as altered aragonite ooids. However, ooids from the Kelloway Rock were originally chamositic²³, and their diagenesis is not relevant to that of aragonitic ooids.

In some units, there is extensive oomouldic porosity that in some cases is a burial diagenetic phenomenon. Such pervasive subsurface oomouldic porosity occurs in the Ste Genevieve but is most strongly developed in the Smackover (Jurassic, Oxfordian, south-central USA). The Smackover varies from oomouldic in the northern zone to very well preserved radial-concentric ooids in the southern zone²¹. If one assumes that there was a north–south change in original mineralogy, then somewhere one could reasonably expect a zone of mixed ooids with two distinct diagenetic behaviours, as in some Upper Carboniferous and Lower Cambrian oolites (Figs 4, 5). This is not the case, and the petrographical properties and facies relationships of the Smackover have been taken (C. H. Moore, personal communication) to indicate that the ooids must have been of the same mineralogy throughout the unit. Accepting

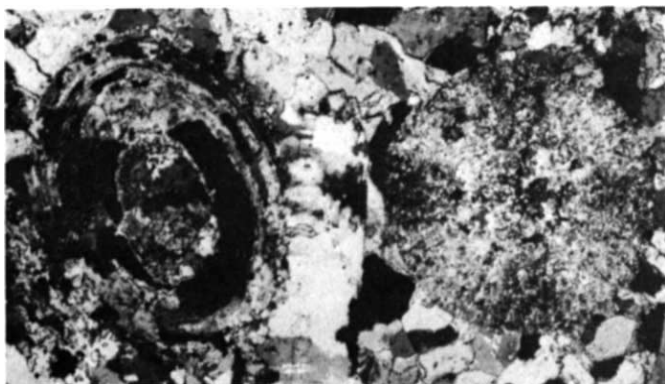


Fig. 3 Ordovician oolite (University Texas, TC 630 00k)^{2,3}, Oklahoma, USA. Thin section, plain light, $\times 100$. The replacement spar contains little relic structure, in contrast to neomorphic spar after known aragonite.

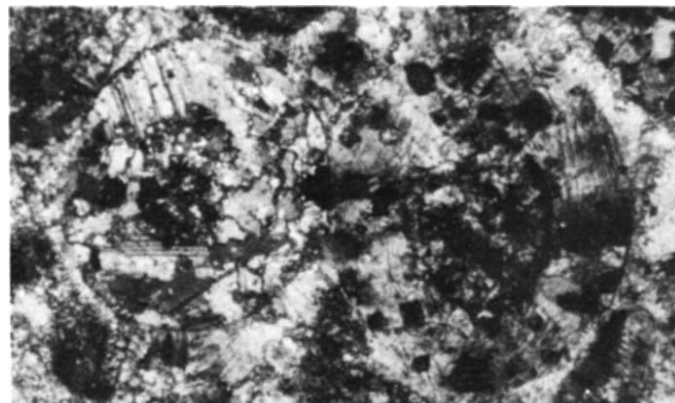


Fig. 5 Early Cambrian oolite (University of Indiana collection, A215), Mural Ls, B.C., Canada. Thin section, crossed nicols, $\times 55$. Comments as for Fig. 4. However, the pseudouniaxial cross in the right-hand (calcitic) ooid is obscured by the coarse radial texture typical of Cambrian ooids and by dolomite rhombs.

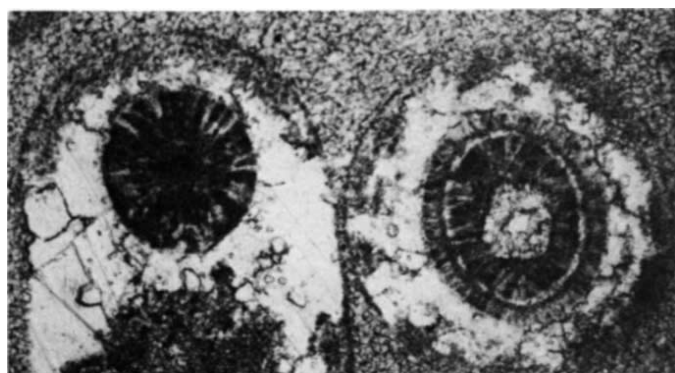


Fig. 4 Pennsylvanian oolite (A. V. Carozzi collection, 6275). Thin section, crossed nicols, $\times 90$. The disrupted ooid on the left is calcitized aragonite; the originally calcitic ooid on the right is still texturally and optically (pseudouniaxial cross) radial.

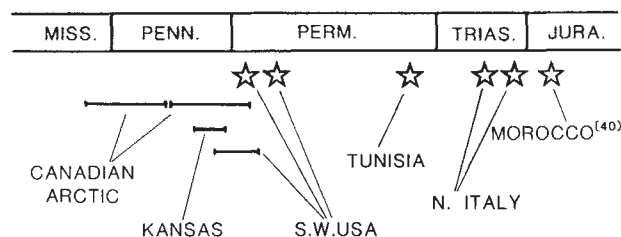


Fig. 6 Temporal and geographical distribution of Phanerozoic, pre-Cenozoic aragonite cements supported primarily by criteria (1)–(3). The early Cambrian aragonite cements⁴⁴ are not included. Sources: Canadian Arctic, refs 33, 34; Kansas, P.A.S and B. N. Popp, in preparation; south-west USA, refs 15, 35, 36; Tunisia, ref. 11; northern Italy, refs 14, 16; Morocco, ref. 10.

this, we are faced with an apparent dilemma. Either precise pseudomorphing of fine radial aragonite ooids by calcite has occurred in the southern zone, or there has been widespread dissolution of originally calcitic ooids in the northern zone. General arguments against pseudomorphic calcitization stated above are equally applicable to the fine-textured ooids of the Smackover southern zone. What about the alternate explanation: dissolution of calcitic ooids? Mouldic preservation of known calcitic skeletons occurs commonly in dolomitized units²⁴, and oomoulds in dolomitic rocks^{25,26} are particularly unsure indicators of ooid mineralogy. Moulds of unquestionably calcitic constituents (such as miliolid foraminifera) can occur in essentially surface conditions in non-dolomitic rocks, in which even some relatively fine, early calcitic cements are dissolved²⁷. The regional scale oomouldic porosity in the Smackover is, if not unique, at least quite unusual.

The difference in mode and apparent timing of dissolution of ooids and aragonitic molluscan remains in the Great Oolite suggest an original calcite mineralogy for those now mouldic ooids. Experimental work²⁸ has demonstrated that, in totally calcitic Ste Genevieve limestone samples, there can still be a selective dissolution of calcitic ooids, apparently as a function of the finer crystal size in the ooids, relative to the surrounding cement. The experimental results are very like naturally occurring mouldic porosity in subsurface samples of the same unit (A. V. Carozzi, personal communication). Incipient mouldic porosity has been encountered even in very fine-textured, morphologically and optically radial calcite ooids in the Smackover southern zone (Walker Creek Field) (C. H. Moore, personal communication, 1982).

The implications of these observations are that: (1) mouldic preservation alone is not a secure criterion for original aragonite mineralogy, and (2) dissolution of fine-textured calcite constituents can occur, predominantly in deep burial environments or associated with near surface leaching.

Comparison of data on ooids and cements

If one eliminates equivocal or doubtful examples (criteria (5) and (6)), then inferred aragonite ooids show a discontinuous temporal distribution (Fig. 2). The oscillating trend implied by the ooid data led me to investigate temporal distribution of inferred aragonite cements. The same criteria for recognition of aragonite discussed earlier are applicable to cements. Various acicular or bladed, often isopachous cements in rocks of diverse ages have been called the product of pseudomorphic calcitization of an acicular aragonite precursor. Such an inference, like the comparable one for ooids, relies heavily on uniformitarian assumptions of mineralogical equivalence with modern acicular aragonite cements. It has been pointed out²⁹ that, for a long time, an apparently widespread view existed that only aragonite produced acicular cements or even submarine cements at all, despite even very early³⁰ observations to the contrary. Recent studies have shown increasingly that high-Mg calcite is common as a bladed or fibrous cement³¹, often as a fairly solid palisade³², which can be morphologically quite similar to coexisting aragonite cements. Radial-fibrous calcite, and its common companion, fascicular-optic calcite, preserve a degree of optical and textural regularity which, if derived from an aragonite

precursor, would require assumption of pseudomorphing. Calcitized examples of botryoidal cements and other aragonite constituents substantiated by criteria (2) or (3) (refs 1, 9, 10, 15, 16, 20 and P.A.S. and B. N. Popp, in preparation) show textural disruption which refutes that assumption. If one excludes such supposed instances of pseudomorphic replacement, then Phanerozoic, pre-Cenozoic once-aragonitic cements (based mainly on criteria (1)–(3) and/or presence of raggioni¹⁶) show a clumped distribution in the geological record (Fig. 6). These 'reliable' aragonite cements include primarily those which are still aragonite^{11,14} (usually encased in later calcite cement), as well as calcitized botryoidal aragonites (or raggioni¹⁶) which usually contain quite a high Sr content^{15,33} and even aragonite relics. Some botryoidal cements accepted here as probably originally aragonite (late Mississippian³⁴, Pennsylvanian–Permian^{35,36} and possibly one early Jurassic [Sinemurian]³⁷) have not been investigated elementally, but are morphologically and texturally like calcitized botryoidal aragonitic cements confirmed by criteria (2) and (3), that is, criterion (4) applies. It is, I believe, significant that, although isopachous, fibrous early marine cements are relatively common in younger Jurassic (late Pliensbachian) to Cretaceous oolitic units and hardgrounds^{38–40}, no verifiable aragonite cements (criteria (1)–(3), or even (4)) appear to have been described from rocks of those ages.

Inferred temporal trends

The ooid and cement data are complementary. Their composite indication is that there have been, in the Phanerozoic, several oscillations above and below an apparent 'aragonite threshold'. The most likely mechanism^{2,5,6} appears to be tectonically induced changes in P_{CO_2} , rather than changes in oceanic Mg/Ca ratio, which various authors have suggested^{41,42} and which I previously favoured¹. Mackenzie and Pigott² presented a model linking elevated P_{CO_2} to 'submergent modes', times of high sea-level stand, high spreading rates and high rates of subduction zone metamorphism. That metamorphism elevated atmospheric P_{CO_2} through reverse weathering reactions⁴³. During times of low spreading rates and low sea-level stand (their oscillatory modes), the reduced metamorphism would result in decreased flux of CO_2 to the atmosphere. In those conditions, it was suggested that aragonite could precipitate, in association with calcites with relatively higher $MgCO_3$ content. Fischer^{5,6} recently proposed a Phanerozoic oscillation between climatic episodes which he characterized as 'greenhouse' (essentially equivalent to the 'submergent modes') and 'icehouse' (equivalent to the 'oscillatory modes') episodes. Fischer sug-

gested two tectonically influenced sources of change in P_{CO_2} : removal by continental weathering, and addition by vulcanism. The influence of those mechanisms, relative to sea-level stand, is in the same sense as the metamorphic mechanism². Fischer^{5,6} noted a temporal correlation between changes in his climatic episodes and the oscillations in the sea-level curves and in vulcanism, as implied by a granite emplacement curve (Fig. 1).

Note that the mechanism implicated^{1,42} for inferred increase in the oceanic Mg/Ca ratio, namely selective removal of calcium by low-Mg calcite plankton (coccoliths and globigerinacean foraminifera) cannot explain the inferred Carboniferous shift (Fig. 1), which predates them. Furthermore, if an increase in oceanic Mg/Ca ratio^{41,42} occurred during mid- and late-Mesozoic time, its influence on non-skeletal carbonate mineralogy would have been counter to the influence of increased P_{CO_2} during that submergent episode. That would presumably have produced a lesser departure (as implied in Fig. 1) from present conditions than would have been true during the comparable Palaeozoic aragonite-inhibiting episode. It is evident that, as is the case today, inorganic aragonite and calcite coexisted in 'aragonite-facilitating' episodes. However, during those episodes, aragonite was apparently always the less common non-skeletal polymorph, as it may well be today.

The oscillating trend suggested at the top of Fig. 1 reflects only inferred first-order changes in non-skeletal carbonate mineralogy. It is possible that second-order oscillations could have resulted in short-term aragonite-facilitating conditions in an otherwise primarily aragonite-inhibiting episode, or vice versa. The difference in conditions between those which facilitate and those which inhibit aragonite precipitation may well not be extreme. Modern ooids¹⁷ in Baffin Bay, Texas, and Pennsylvanian ooids (P.A.S. and B. N. Popp, in preparation) from several units in Kansas are bimineralic, reflecting essentially contemporaneous differences in environmental controls on mineralogy. Although the P_{CO_2} mechanism seems well supported circumstantially, there is a need for experimental work to investigate that mechanism, as well as the possible synergistic or antithetical influence of other factors, such as differences in dissolved Mg/Ca ratio.

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Genomic substitutions of centromeres in *Saccharomyces cerevisiae*

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The centromere region of yeast chromosome III has been investigated by altering it in vivo. Deleting the functional centromere (CEN3) sequence leads to extreme instability of the resulting acentric chromosome. Inversion of CEN3, or its replacement by chromosome XI centromere DNA (CEN11) has no measurable effect on the mitotic and meiotic behaviour of chromosome III, suggesting that yeast centromeres are not chromosome-specific, and are fully functional in either orientation.

THE stable transmission and faithful segregation of the eukaryotic chromosome through the intricate cellular processes of mitosis and meiosis depend on proper centromere function. In addition to providing sites for attachment of spindle fibres, the centromeres of sister chromatids must perform the dual role of remaining joined in meiosis I and splitting in meiosis II. Centromeric DNA segments from several chromosomes of *Saccharomyces cerevisiae* have been cloned, identified and characterized¹⁻⁴. The nucleotide sequences of *CEN3*, *CEN11* (ref. 5) and *CEN6* (ref. 4) have been determined, and the similarities among the three include an extremely A+T-rich region 87–89 base pairs (bp) in length (element II), immediately followed by an 11-bp region of nearly perfect homology (element III). *CEN3* and *CEN11* also have another 14-bp sequence of perfect homology (element I) directly preceding the A+T-rich region.

Using assays based on mitotic stabilization of autonomously replicating *ARS* plasmids in yeast, as well as 2+:2– segregation of the plasmids through meiosis, we have localized centromere DNAs from yeast chromosomes III and XI to 627-bp and 858-bp segments of chromosomal DNA, respectively⁵. The plasmid-mediated assay for definition and characterization of proper centromere function has several serious drawbacks, however, as the mitotic and meiotic behaviour of the small minichromosomes containing centromere sequences does not always faithfully mimic that of the larger parental chromosomes^{1,2}.

A preferable approach towards examining centromere function *in vivo* is to make sequence alterations, deletions or substi-

tutions directly in the normal, parental yeast chromosome. Such operations are readily carried out using a recently described procedure that permits DNA sequence conversion by fragment-mediated transformation⁶. We have constructed a centromere-targeted alteration vector that contains, on a single restriction fragment, sequences flanking the 627-bp *CEN3* region of chromosome III and a unique restriction site and selectable marker between these flanking sequences. This vector has permitted us to introduce a number of alterations directly into the centromere region of yeast chromosome III *in vivo*.

These alterations include deletion of the entire 627-bp *CEN3* region, introduction of the *CEN3* sequence into chromosome III in the reverse orientation, and the substitution of the 858-bp *CEN11* sequence for that of *CEN3* in both the correct and reverse orientations. The deletion of the entire 627-bp *CEN3* region results in a dramatic instability of chromosome III. The other substitutions, all of which interrupt the DNA coding sequence on either one or both sides of *CEN3*, have no measurable effect on the mitotic and meiotic behaviour of chromosome III *in vivo*. These observations lead to the conclusion that yeast centromeres are not necessarily chromosome specific.

Fragment-mediated transformations

A method has recently been described which allows the alteration or deletion of a specific region of the yeast genome by a single transformation with an appropriate DNA fragment⁶. The method is based on the observation that free DNA ends are extremely recombinogenic during transformation and interact directly with homologous sequences in the genome. A portion

Table 1 Meiotic behaviour of chromosome III containing substituted *CEN* regions

<i>a/a</i> Diploid strain	Apparent map distance (centimorgans)			Tetrads scored	Spore viability (%)	% Gene conversions		
	<i>HIS4-LEU2</i>	<i>LEU2-URA3</i>	<i>URA3-MAT</i>			<i>HIS4</i>	<i>LEU2</i>	<i>MAT</i>
303-4/ <i>CEN3</i>	14	4	29	24	96	12.5	4	0
303-6/ <i>CEN3</i>	21	10	34	22	94	4.5	9	0
311-9A/ <i>CEN3</i>	25	6	34	34	100	6	0	0
311-9B/ <i>CEN3</i>	21	5	55	56	97	18	1.8	3.6
311-11/ <i>CEN3</i>	21	2	19	27	95	15	0	0
311-11/311-11 (SB11f1)	15	—	42	27	80	15	11	0
			(<i>LEU2-MAT</i>)					
Literature values	9.9–19.9	3.4–11.6	20–23.1	—	—	8.4	2.5	0.6
		(<i>LEU2-CEN3</i>)	(<i>CEN3-MAT</i>)					

The various *a/a* diploids were constructed by transformation of strain SB9882-4 (*a trp1-289 ura3-52 leu2-3 leu2-112 his4-519/a trp1-298 ura3-52 can1*) with the centromere-substitution *EcoRI* fragments as described in the text. Strain SB11f1 (*a URA3⁺ leu2 his4 trp1 can1/a URA3⁺ LEU2⁺ HIS4⁺ trp1 can1*) was obtained by mating two haploid *URA3⁺* progeny from the 311-11/*CEN3* tetrads. The apparent map distances were calculated from recombinational frequencies as described by Mortimer and Hawthorne¹⁵. Gene conversions were scored as the percentage of 3+:1– plus 1+:3– tetrads for the marker being scored. All tetrads were 2+:2– for the *ura3* marker (except for SB11f1, where all were 4+:0–). Literature values are taken from refs 15, 16.

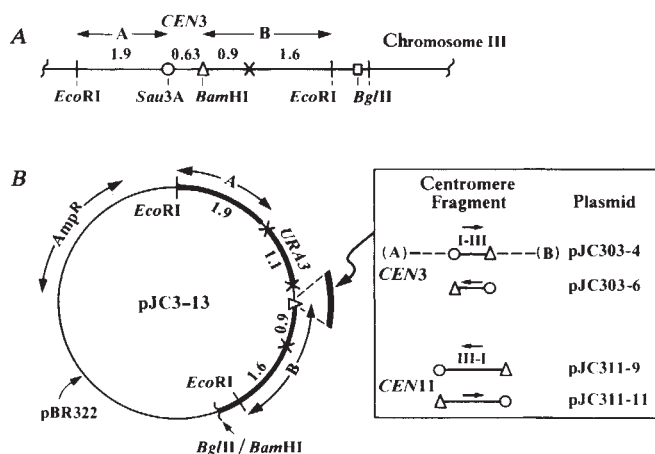


Fig. 1 **A**, Physical map of the centromere region of *S. cerevisiae* chromosome III, giving the location of *EcoRI* (+), *BamHI* (Δ), *HindIII* ($\times$), *BglII* ($\square$) and pertinent *Sau3A* ($\circ$) sites. Numbers denote kilobase pairs (kbp). The locations of the 627-bp *CEN3* *Sau3A*-*BamHI* sequence, and of the 1.9-kb flanking region A and the 2.5-kb flanking region B are as indicated^{1,5}. **B**, Physical map of the centromere substitution vector, pJC3-13. Plasmid pJC3-13 was constructed by first inserting a 1.1-kbp *HindIII* fragment of yeast DNA carrying the *URA3* gene¹⁸ into the *HindIII* site of pBR322. A 1.9-kbp *EcoRI*-*Sau3A* fragment from the left centromere flanking region (A) of chromosome III was then ligated into the single *EcoRI* site, maintaining the *EcoRI* site only at the leftmost end of the fragment. Finally, a 2.9-kbp *BamHI*-*BglII* restriction fragment from the right centromere flanking region was inserted into the single *BamHI* site of the plasmid above, regenerating a single *BamHI* site in the middle of the two flanking regions. The 2.9-kbp *BamHI*-*BglII* fragment also contains a single *EcoRI* site as shown. All recombinant DNA methodology was as previously described¹. The map gives the locations of centromere III flanking regions A and B, the *URA3* gene, the unique *BamHI* site and the two *EcoRI* sites which delineate the boundaries of the centromere substitution fragment. The table to the right of the map shows insertions of the 627-bp *CEN3* *Sau3A*-*BamHI* fragment or the 858-bp *CEN11* *Sau3A*-*BamHI* fragment in either orientation into the unique *BamHI* site of the vector to yield the plasmids, pJC303-4, pJC303-6, pJC311-9 and pJC311-11 as indicated. Roman numerals and arrows above the fragments give the orientations of centromere sequence elements I, II and III (ref. 5).

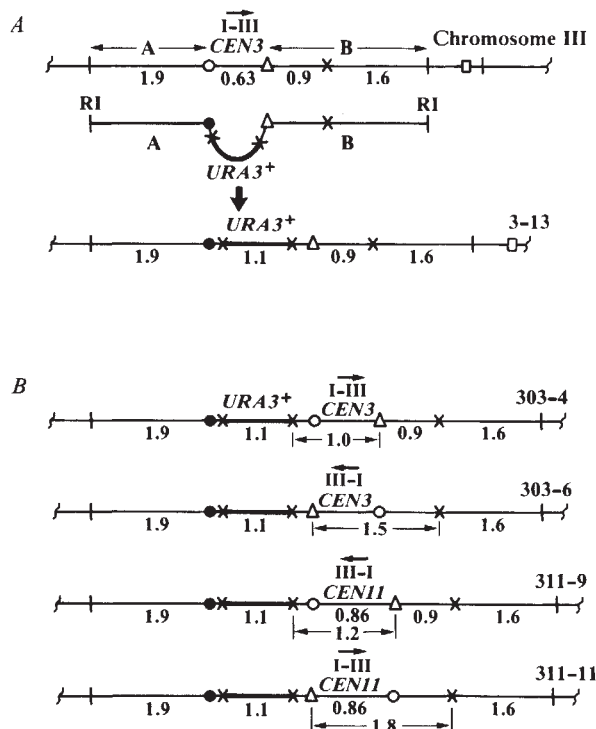


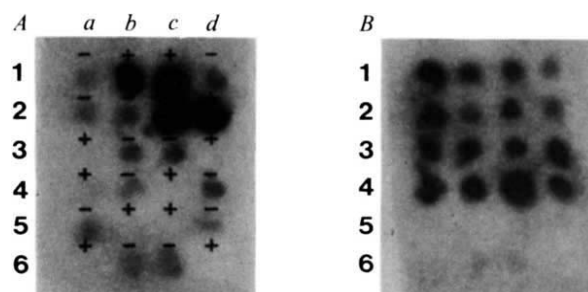
Fig. 2 **A**, Schematic representation of conversion of the centromere III region of chromosome III to the acentric 3-13 construction. Transformation of a diploid yeast strain with *EcoRI*-restricted pJC3-13 DNA is expected to yield *Ura*⁺ transformants in which one, or, at a much lower frequency, both centromere III regions are converted to those sequences carried by the centromere-substitution *EcoRI* fragment⁶. In the case of pJC3-13, the fragment-mediated transformation results in the substitution of *URA3* for *CEN3*. **B**, Transformation with *EcoRI*-restricted pJC303-4, pJC303-6, pJC311-9 and pJC311-11 DNAs yields diploid transformants in which one copy of the *CEN3* sequence is replaced by *URA3* plus either *CEN3* in the proper genomic orientation (303-4 construction), with sequence elements I, II and III oriented from left to right, in the direction of *LEU2* to *MAT* on the genetic map; *CEN3* in the opposite orientation from that found normally in chromosome III (303-6 construction); the 858-bp *CEN11* sequence, with its elements I, II and III oriented as they are in genomic *CEN3* (311-9 construction); or finally, *CEN11* replacing *CEN3* in the reverse sequence orientation (311-11 construction). All yeast transformations were carried out according to Hinnen *et al.*¹⁹ with *EcoRI*-restricted plasmid DNAs that had undergone a single phenol extraction and precipitation from 70% ethanol.

of a yeast chromosome can, therefore, be directly converted to a desired alternative sequence, provided that the fragment used for transformation carries the modified sequence and a selectable genetic marker, flanked on either side by the homologous sequences normally found in the genome (see, for example, Fig. 2A). To modify the centromere region of chromosome III, we have constructed a centromere substitution vector. Restriction maps of this plasmid vector, pJC3-13, and the pertinent region of chromosome III are shown in Fig. 1. Vector construction is described in the legend to the figure. Plasmid pJC3-13 contains genetic information found directly to the left (flanking region A, 1.9 kbp) and to the right (flanking region B, 2.5 kbp) of the 627-bp *CEN3* region, a selectable marker (*URA3*) and a unique *BamHI* cloning site, located within a small region of pBR322 DNA, for insertion of desired sequences between the two flanking regions. This construction can be released from the plasmid on a single *EcoRI* fragment whose ends are homologous with those sequences flanking both sides of centromere III.

The 627-bp *CEN3* *Sau3A* fragment from yeast chromosome III and the 858-bp *CEN11* *Sau3A* fragment from chromosome XI (ref. 5) were inserted into the single *BamHI* site of pJC3-13 in both orientations, yielding the four plasmid constructions illustrated in Fig. 1B. These plasmids and pJC3-13 were restricted with *EcoRI* and 10 μ g of each DNA were used to transform the *ura3* diploid yeast strains SB9882-4 (see Table 1 legend for genotype) or SB9882-4CR (contains the recessive cryptopleurine-resistance allele, *cry1*) to the *Ura*⁺ phenotype, resulting in each case in the substitution of the 627-bp *CEN3* sequence in one of the chromosomes III of the host with those sequences carried by the fragment. It was anticipated that the structures of the altered centromere III regions of the transformants would fall into the five categories illustrated and described in Fig. 2.

Diploid strains were chosen as hosts for all the transformations because an altered chromosome III, lacking a centromere, or in other cases, possessing the wrong centromere or the correct centromere in the wrong orientation, might be very unstable

Fig. 3 A, Autoradiogram of colony hybridization with a ^{32}P -labelled *CEN3* probe to various meiotic tetrads. The letters *a*, *b*, *c* and *d* refer to the four meiotic products of a single tetrad in each row. Rows 1 and 2 are two representative tetrads derived from the sporulation of a stable *URA3*⁺ diploid transformant obtained using pJC3-13 DNA. As this particular transformant was stable for the *Ura*⁺ phenotype (after non-selective growth; see text), and *URA3* was linked to *LEU2* on chromosome III, it was necessary to determine if all meiotic progeny from the transformant contained *CEN3* sequences. Although *CEN3* sequences are clearly present in all four haploid progeny in each case, the strongest hybridization is seen with the *Ura*⁺ sister haploids, which may indicate the mechanism by which *URA3* has become stabilized in at least some of the aberrant transformants. A likely mechanism would involve integration of uncut pJC3-13 by homologous recombination into chromosome III¹⁹ or integration of a pJC3-13 molecule that was cut only once by *EcoRI*²⁰. Either event would introduce the entire pBR322 sequence into the chromosome along with the *URA3* gene. As our *CEN3* probe also contains some pBR322 sequences, it would show stronger hybridization with those colonies that contain more pBR322 DNA. Rows 3 and 4 represent two tetrads from the sporulation of a diploid carrying the 311-9 construction, and rows 5 and 6, the 311-11 construction. In all transformations carried out with *CEN*-containing fragments, transformation frequencies were high (>1,500 transformants per μg DNA) and all the transformants examined carried the conversion in one copy of chromosome III that is predicted from the structure of the original transforming fragment. *URA3*(+) and *ura3*(-) sister spores for each tetrad are indicated on the diagram. **B**, Autoradiogram of colony hybridization with a ^{32}P -labelled *CEN3* probe to haploid transformants carrying altered centromere III regions. Haploid yeast strain SB12982 (*a ura3-52 trp1-289 leu2-3, 112 his4-519 cry1*) was transformed, as described in Fig. 2 legend, with pJC3-13, pJC303-4, pJC303-6, pJC311-9 and pJC311-11 DNAs restricted with *EcoRI*, selecting for *Ura*⁺ transformants. Transformation frequency was low (200 transformants per μg DNA) for *EcoRI*-cut pJC3-13 DNA and high (>2,000 transformants per μg DNA) for the *CEN*-containing fragments. Rows 1 and 2 are transformants picked at random from the pJC3-13 transformation; these have varying growth rates and all contain *CEN3* sequences. It is, therefore, assumed that haploid strains with an acentric chromosome III do not survive. The remaining rows (3-6) represent transformants picked at random from transformations with *EcoRI*-restricted pJC303-4, pJC303-6, pJC311-9 and pJC311-11 DNAs, respectively. As anticipated from the structures of the transforming fragments, transformants obtained using pJC303-4 and pJC303-6 DNAs contain *CEN3* sequences (rows 3 and 4), whereas those derived from pJC311-9 and pJC311-11 fragments do not (rows 5 and 6). Colony hybridizations were carried out according to Tschumper and Carbon²¹, except that hybridization conditions were those of Reed²². Approximately 1.5×10^6 c.p.m. of nick-translated²³ 627-bp *CEN3* probe were used per filter.



mitotically, as spindle fibre attachment may be affected. In addition, each of the alterations or replacements interrupts the normal coding sequence on one or both sides of the genomic 627-bp *CEN3* sequence and could inactivate essential gene sequences, which would then be provided by the other copy of chromosome III in the diploid host.

Acentric chromosome III

When the diploid strains SB9882-4 or SB9882-4CR were transformed with *EcoRI*-restricted plasmid pJC3-13 DNA, 250-300 transformants per μg DNA were obtained on selection for the *Ura*⁺ phenotype. In previous experiments, only a few transformants were obtained per μg of uncut plasmid pJC3-13, indicating the absence of an *ARS* sequence on flanking regions A or B. The *Ura*⁺ transformants exhibited a variety of colony sizes, with only about 10% of the total appearing after 36 h, and the rest after 3-4 days at 32 °C. This transformation frequency and rate of colony appearance are considerably less than are seen in transformations with *CEN*-containing fragments or with *ARS*-containing plasmids such as *Yrp7* (ref. 7). Further analysis of 80 of the pJC3-13-derived transformants revealed that ~37% of the total (both fast and slow growers) were stable for the *Ura*⁺ phenotype after many generations of non-selective growth. The remaining 63% of the transformants (all relatively slow growers on selective medium) were extremely unstable for the *Ura*⁺ phenotype, and after 10 generations of non-selective growth, the *Ura*⁺ phenotype is lost in ~90% of the cells.

Over half of the *Ura*⁺-unstable class of transformants (or 33% of the total) were of the two types expected for fragment-mediated replacement of one of the genomic copies of the *CEN3* sequence by the *URA3* gene in the diploid host (see Fig. 2A). That is, one type of transformant, in which the transforming fragment had presumably substituted into the *his4 leu2 MATa* copy of chromosome III, was, after 10-15 generations on non-selective medium, still phenotypically *His*⁺ *Leu*⁺ but mostly *Ura*⁻, and those individual clones that had lost the *Ura*⁺ phenotype simultaneously became fertile (*MATa*). Deletion of a centromere from one copy of chromosome III causes

that chromosome to become very unstable and to be lost rapidly from the population, thus uncovering the *MATa* locus on the remaining copy of chromosome III. The other type of transformant, that in which the transforming fragment had substituted into the *HIS4 LEU2 a* chromosome, loses this chromosome with high frequency. Most individual clones in this group, after 10 generations of non-selective growth, become *Ura*⁻ and the *his4* and *leu2* markers on the left arm and the *MATa* marker on the right arm of the remaining copy of chromosome III are simultaneously uncovered.

Two diploid transformants with both a normal and an acentric copy of chromosome III were examined in greater detail. Even in selective growth conditions, 60-80% of the individual cells in populations of these two transformants are *Ura*⁻ and have thus lost the acentric chromosome (based on the appearance of recessive markers on both arms of the remaining chromosome III). When selective pressure is removed by 10 generations of growth on non-selective medium, only a small fraction of the individual cells (5-9%) are still *Ura*⁺. The generation time of each of the two transformants in liquid yeast extract-peptone-glucose (rich) medium is 2.2 h at 32 °C, which is somewhat longer than the 1.6-h generation time of the parental diploid strain SB9882-4 and is perhaps indicative of the $2n-1$ chromosomal complement in these cells.

The remaining transformants of the *Ura*⁺-unstable class (30% of the total) were *Ura*⁻, *Leu*⁺, *His*⁺, and sterile after prolonged growth on non-selective medium. This phenotype is not consistent with the predicted conversion mediated by fragment transformation, and arose by an as yet undetermined alternative mechanism.

Several of the *URA3*-stable clones from the transformation with the pJC3-13 fragment were also examined in greater detail. Two of these diploids were sporulated and the resulting asci were dissected for genetic analysis. One transformant yielded asci with two viable and two non-viable sister spores. The viable haploid progeny were *Ura*⁻ in all the tetrads examined and contained either of the two normal copies of chromosome III (data not shown). Thus, in this transformant the transforming fragment is probably stably incorporated into a chromosome other than III in such a way that it has inactivated an essential

sequence. The other *URA3*-stable transformant yielded four viable spores in its asci, and in each tetrad, *URA3* was linked to the *LEU2* marker and the centromere on chromosome III. It was possible, however, to demonstrate by colony hybridization using a *CEN3* DNA probe that the *URA3*-stable transformants still contain two copies of *CEN3*, and thus represent an aberrant class in the population of transformants (Fig. 3A).

Thus, deletion of the 627-bp *CEN3* sequence from chromosome III results in dramatic chromosomal instability. We could not obtain haploid strains containing an acentric chromosome III, either by the fragment-mediated transformation of a haploid or by sporulation of the 3-13 diploid. The presence of an actively transcribed *URA3* gene in the acentric constructions and the interruption of the coding sequence around *CEN3* are not responsible for the instability, as is demonstrated below. Therefore, various *in vitro*-produced alterations of the centromere regions can readily be assayed for functional changes within the parental chromosome by this type of fragment-mediated conversion. The ease with which acentric chromosomes are generated in these experiments provides a unique opportunity for their study. In other eukaryotes, acentric chromosomes are also unstable, but in *Neurospora*, for example, certain fragments do persist and replicate synchronously with the centric chromosomes^{8,9}.

Inversion and replacement of *CEN3*

When yeast strains SB9882-4 or SB9882-4CR were transformed with *Eco*RI-restricted plasmid pJC303-4, pJC303-6, pJC311-9 or pJC311-11 DNAs, 1,000–3,000 *Ura*⁺ transformants were obtained per µg of DNA. These transformants appeared within 24–36 h at 32 °C; the parent diploid and individuals of each transformant class have an identical generation time at 32 °C in rich medium of 1.6 h, and the *Ura*⁺ phenotype is extremely stable in all transformants (see below).

Four transformants, derived in SB9882-4 and carrying each one of the four centromere alterations or replacements, were sporulated, and resulting asci were dissected for meiotic analysis. At least 25 tetrads were analysed for each class, and the resulting haploid products were examined both genetically and biochemically. As was expected from the method of construction, the *URA3*⁺ marker in the transformants was closely linked to the *LEU2* locus, which is near the centromere on chromosome III (see Table 1).

Direct evidence to support the genomic structures as represented in Fig. 2B was obtained by two types of hybridization experiments. An autoradiogram of a colony hybridization experiment with haploid progeny of various tetrads from the meiotic analyses (Table 1), using the *CEN3* 627-bp *Sau*3A fragment as probe, is presented and described in Fig. 3A. In tetrads from each of the two classes of transformants in which *CEN3* is replaced by *CEN11*, only those sister spores that are *Ura*⁺, and thus have an unaltered copy of chromosome III, contain *CEN3* sequences. The remaining two (*Ura*⁺) sister spores in each ascus lack these sequences entirely. Hybridizations to haploid transformants carrying the altered centromere constructions yield analogous results (Fig. 3B).

To confirm more precisely that the alterations in the various transformants are indeed those predicted from the structures of the transforming fragments, Southern blot hybridization experiments¹⁰ were carried out on total genomic DNA preparations of two *Ura*⁺ haploid meiotic progeny (of both the α and α mating types), picked at random from tetrads generated in the meiotic analyses of the four types of diploid transformants. A Southern blot hybridization, in which DNAs of the haploid progeny as well as control wild-type and plasmid DNAs were restricted with a combination of *Bam*HI and *Hind*III endonucleases, electrophoresed on a 1% agarose gel, blotted to a nitrocellulose filter and probed with ³²P-labelled *CEN3* 627-bp fragment, is shown in Fig. 4A. The *CEN3*-containing plasmids, pJC303-4 and pJC303-6, each contain single *Bam*HI–*Hind*III fragments of 1.0 and 1.5 kb, respectively, that hybridize to the

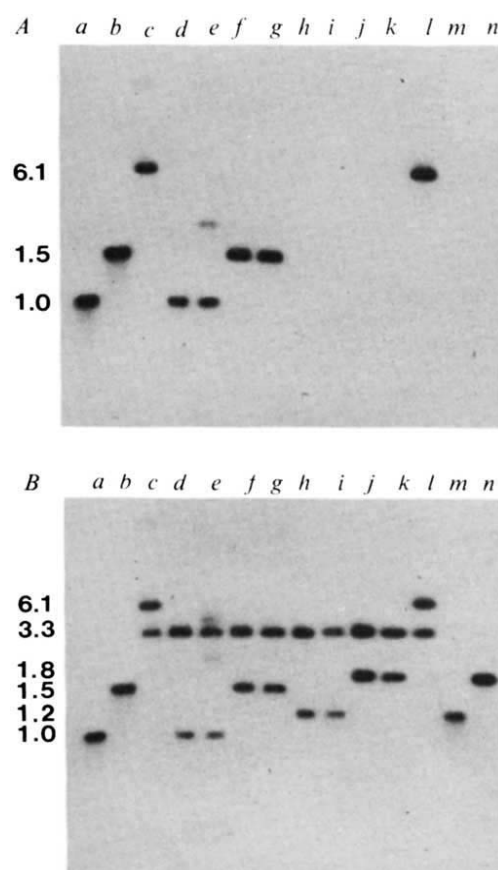


Fig. 4 **A**, Southern blot hybridization autoradiogram of total yeast DNAs from haploid meiotic progeny of various transformants with ³²P-labelled 627-bp *CEN3* fragment as probe. All DNAs were restricted with a combination of *Bam*HI and *Hind*III. Lanes a, b, m and n contain control plasmid pJC303-4, pJC303-6, pJC311-9 and pJC311-11 DNAs, respectively, and lanes c and l, total genomic DNA from strain SB9882-4. Lanes d–k represent two haploid *Ura*⁺ meiotic progeny from sporulation and asci dissections of diploid transformants carrying the 303-4, 303-6, 311-9 and 311-11 constructions, in that order. Preparation of DNAs, conditions of blotting, and hybridization to ³²P-labelled 627-bp *CEN3* probe were exactly as described in ref. 1. **B**, The same Southern blot as in **A**, probed with a mixture of the *CEN3* probe plus the ³²P-labelled 858-bp *CEN11* DNA fragment.

CEN3 probe (lanes a and b, Fig. 4A; see Fig. 2B for restriction maps). The wild-type genomic *CEN3* sequence is contained on a 6.1-kb *Bam*HI–*Hind*III fragment (lanes c, l, Fig. 4A and ref. 1). Only those haploid DNAs derived from *URA3*⁺ transformants carrying the 303-4 (lanes d, e) and 303-6 (lanes f, g) constructions hybridize to the *CEN3* probe, visualizing *Bam*HI–*Hind*III fragments that are identical in size to their respective plasmid fragments. As expected, no fragment corresponding to the genomic *CEN3* band is seen in any of the lanes, except those of the control wild-type DNA.

When the same Southern blot filter is hybridized to a combination of *CEN3* and 858-bp *CEN11* probes (Fig. 4B), additional bands corresponding to the *Bam*HI–*Hind*III fragments from pJC311-9 (1.2-kb fragment, lane m) and pJC311-11 (1.8 kb fragment, lane n) are seen for those haploid DNAs generated from transformants carrying the 311-9 (lanes h, i) and 311-11 (lanes j, k) constructions. As expected, bands corresponding to the 3.3-kb genomic *Hind*III fragment of chromosome 11 that contains *CEN11*⁵ are present in all the genomic DNA lanes. Blot hybridizations carried out with *Eco*RI plus *Bam*HI-restricted DNAs from the original diploid transformants and a combination of *CEN3* and *CEN11* probes (data not shown) also confirm that the configurations of the *CEN3*

Table 2 Mitotic gene conversion, recombination and chromosome III loss in diploid strains with altered centromere III constructions

Sequences at centromere III of diploid	Frequency (per cell division) of mitotic gene conversion and recombination at <i>MATa</i> locus, including chromosome III loss	Frequency (per cell division) of mitotic gene conversion and recombination at <i>cry1</i> locus
303-4/ <i>CEN3</i>	0.9×10^{-5}	2.7×10^{-5}
303-6/ <i>CEN3</i>	0.3×10^{-5}	2.9×10^{-5}
303-9/ <i>CEN3</i>	1.1×10^{-5}	2.7×10^{-5}
311-11/ <i>CEN3</i>	0.6×10^{-5}	2.4×10^{-5}
<i>CEN3/CEN3</i> (SB9882-4)	0.7×10^{-5}	—
<i>CEN3/CEN3</i> (SB9882-4CR)	—	2.4×10^{-5}
311-11/311-11 (SB11f1)	1.0×10^{-5}	—

Quantitative mating experiments to determine the frequency (per cell division) of mitotic gene conversion and recombination, including chromosome III loss, in cultures of each of the four transformant classes listed above, in a control culture of diploid SB9882-4, and a culture of SB11f1 (see Table 1 legend) were carried out as described by Dutcher *et al.*¹⁷. Approximately 3×10^6 cells of the control diploid or the transformants, all of which were derived in strain SB9882-4 (Table 1 legend), were mixed with 4.5×10^6 cells of the haploid strain 262 (*thr MATa*). A haploid of the *a* mating type was chosen for these experiments, because it was known from the meiotic data described above that, in the four transformants examined, the altered centromere fragment (carrying the *URA3* marker) had replaced the *CEN3* region in the *MATa* chromosome. Therefore, it is the loss of this copy of chromosome III that is of interest in these experiments. Cells were allowed to mate for 4 h on rich (yeast extract-peptone-glucose) media, then plated onto minimal salts-glucose media lacking threonine and tryptophan. The number of colonies was determined after growth for 48 h at 32 °C. Duplicate samples were taken for each culture and the averages are presented in the table. The transformants used are the same ones for which meiotic data are given in Table 1. The experimental value of one failure in $\sim 10^5$ cell divisions is slightly lower than that reported for chromosome loss in yeast¹¹, because the efficiency of mating and final plating are not optimal in these experiments. The frequency (per cell division) of mitotic gene conversion and recombination at the locus on chromosome III specifying resistance to the antibiotic, cryptopleurine, was determined in control cultures and transformants of SB9882-4CR (+/*cry1*) by plating a known number of cells directly onto rich media containing 1.6 mg ml^{-1} cryptopleurine. Colonies were counted after incubation for 48 h at 32 °C. Determinations were made for five individual clones of each strain. Numbers fluctuated very little, and the median number is presented.

regions in the transformants are those predicted from the structures of the transforming fragments and shown in Fig. 2B.

Meiotic behaviour

Table 1 gives detailed analysis of the meiotic behaviour of the chromosomes III with altered or substituted centromeres. Spore viability for all these analyses was very high (80–100%); thus, in a high percentage of the tetrads, all four haploid products survived meiosis. Because two sister spores of each tetrad are expected to carry one of the centromere III replacements, the alterations are obviously tolerated in the haploid progeny, and in the case of the 311-9 and 311-11 constructions, the total absence in half the haploids of a *CEN3* sequence does not prevent proper chromosome III segregation, nor inhibit growth of the meiotic products. From the frequencies of meiotic recombination, the map distances between various markers residing on chromosome III in the transformants were calculated. The distances between *HIS4* on the left arm and *LEU2* near the centromere on the left arm appear normal (14–21 centimorgans) and are very close to the published values of 9.9–19.9 centimorgans, which differ somewhat with different strains (Table 1). As expected, *URA3* is tightly linked to *LEU2* in all of these transformants and is thus centromere-linked on

chromosome III. The calculated distances between *URA3* or *LEU2* and the mating type locus (*MAT*) on the right arm of chromosome III also fall within published values, with the possible exception of the transformant in which *CEN3* is replaced by *CEN11* in the reverse orientation. In the case of one 311-9 diploid in which over 50 tetrads were analysed, the distance between *LEU2* or *URA3* and *MAT* is somewhat greater (55 centimorgans) than previously published values for the *LEU2*–*MAT* interval. Thus, it is possible that the *CEN11* sequence affects the rate of meiotic recombination to a different extent from *CEN3*.

The numbers of gene conversions at the various loci along both arms of chromosome III also fell within the normal range for all four types of transformants (Table 1). Conversions at *HIS4* were relatively high in comparison with those at *LEU2* or *MAT*, in agreement with previously reported values.

These data indicate that the presence of a different centromere, or the same centromere in the reverse orientation on one of two copies of chromosome III, does not seem to affect proper segregation of chromosomes in a cell undergoing the complicated processes of meiosis, including meiotic pairing, crossing over and gene conversion. Finally, to demonstrate unequivocally that centromeres are not necessarily chromosome specific, two of the haploid products of opposite mating type derived from the transformant in which *CEN3* was replaced by *CEN11* (311-11 construction) were crossed, and the resulting diploid (SB11f1) was sporulated and subjected to meiotic analysis. The measured map distances and meiotic conversion events along both arms of chromosome III are also normal in this strain, which totally lacks *CEN3* sequences (Table 1).

Mitotic analyses

The fidelity with which mitotic yeast cells replicate and segregate their chromosomes has been accurately measured, and the frequency of mitotic chromosome loss is about one failure in 5×10^4 cell divisions¹¹. The frequency of mitotic gene conversion of a particular marker varies with the marker, but is about one event in 10^4 cell divisions, and the frequency of mitotic recombination is lower by roughly an order of magnitude¹². The mitotic behaviour of the altered chromosomes III in the four types of transformants was examined in three ways. First, two representative transformants of each type were grown non-selectively for approximately 20 generations in media containing uracil. Between 1,500 and 2,500 clones of each were then scored for the presence of the *Ura*⁺ phenotype. No loss of *URA3*⁺ was seen with any of the clones. In the same conditions, the *ARS*-containing plasmid, *Yrp7*, is lost in 95% of the individual clones examined. Thus, the centromere-modified copy of chromosome III in all four types of transformants is mitotically very stable.

To examine more accurately the stability of chromosome III in the strains with altered centromere III structures, a second type of experiment was carried out. The number of mating-competent cells was determined in populations of the four transformants for which meiotic data had been obtained, in the SB11f1 (311-11/311-11) diploid, and in the parental diploid, SB9882-4. The measurement of the number of fertile cells appearing in an *a/a* diploid culture is the sum of three separate events: (1) loss of a copy of chromosome III and the simultaneous uncovering of a hemizygous *MAT* locus, (2) gene conversion or homozygosis at the *MAT* locus, and (3) mitotic recombination between the centromere of chromosome III and *MAT* at the four strand stage. Quantitative mating determinations were carried out as described in Table 2 legend, and the measurements are summarized in the table. The results indicate that there is no difference in the frequency of chromosome III loss (plus gene conversion and mitotic recombination) among the transformants of all four types, the diploid SB11f1 and the parental diploid. The mitotic stability of chromosome III is, therefore, unaltered by the various replacements in the centromere region.

A third experiment to examine mitotic parameters was carried out on transformants having each of the four altered centromere constructions. For these experiments the transformants were derived in the diploid strain SB9882-4CR, which carries a recessive, cryptopleurine-resistance marker, located on chromosome III approximately 2.1 centimorgans from the *MAT* locus¹³. Diploid strains carrying *cry1* in the hemizygous state are not resistant to the antibiotic (L.C. and J.C., unpublished); therefore, measurement of the number of cryptopleurine-resistant cells in a population determines only the frequency of gene conversion at that locus plus mitotic recombination, and not the frequency of chromosome III loss. The number of cryptopleurine-resistant cells was measured in five independent clones of each type of transformant and SB9882-4CR. For all four transformants and the parent strain the frequency of gene conversion plus mitotic recombination is approximately one event in 4×10^4 cell divisions (Table 2).

In conclusion, measurements of a number of mitotic parameters indicate no detectable instability or abnormal behaviour of chromosome III induced by the four alterations made in the centromere region, although very minor differences in mitotic behaviour among the various classes cannot be ruled out. Thus, the 627-bp *CEN3* sequence or the 858-bp *CEN11* sequence is sufficient to stabilize chromosome III mitotically in either orientation, regardless of interruptions in the coding sequences on either side.

Discussion

When the 627-bp *CEN3* or the 858-bp *CEN11* sequence is present on a plasmid that contains a yeast chromosomal replicator, that plasmid functions mitotically and meiotically in a manner similar to an ordinary yeast chromosome^{1,2,5}. The behaviour of a minichromosome does not exactly mimic that of the larger, parental chromosome, however. Although the minichromosome is much more stable mitotically than an *ARS* plasmid without *CEN*, it is orders of magnitude less stable than an average yeast chromosome⁵. Furthermore, a minichromosome usually segregates 2+ : 2- in meiosis, but in as many as 50% of the asci either segregates 4+ : 4- or does not appear in the haploid progeny^{1,2,5}. The addition of larger flanking regions of genomic DNA to both sides of a *CEN* sequence on an *ARS* plasmid has very little overall effect on the behaviour of the minichromosome^{1,2,5}.

A more accurate way to define and characterize centromere structure-function relationships is to do so directly in a normal yeast chromosome. Changes in the centromere region of a yeast chromosome are easily obtained by making the desired alterations to the cloned structure *in vitro*, and through fragment-mediated transformation⁶, replacing the normal centromere region with the altered one. We have chosen this approach here because it avoids the problems inherently present in the plasmid assay system.

The removal of the small 627-bp *CEN3* sequence from chromosome III results in dramatic instability of the chromosome. This observation does not prove that this sequence contains all of centromere III, but it does argue that it is an

extremely important element for proper mitotic stability or spindle attachment. The other alterations described above also indicate that the *CEN3* or *CEN11* sequences are sufficient for proper centromere function, as these centromere elements operate in a normal fashion when isolated from surrounding sequences in the chromosome. In the case of all the *CEN*-containing constructions, the coding sequence on one or both sides of the 627-bp region is interrupted, resulting in no measurable effect on proper centromere function. These interruptions include the very close proximity of the *URA3* gene and its active transcription, as well as the interruption of a transcript that crosses the *Bam*HI site marking the left end of flanking region B (Fig. 1A; E. Yeh and J.C., unpublished results).

The fidelity of replication and segregation of chromosome III is unaltered by the insertion of *CEN3* in the reverse orientation, or the replacement of *CEN3* with the *CEN11* sequence in either orientation. There is no measurable change mitotically in crossing over or gene conversion events on chromosome III or in the generation time of strains carrying the various alterations. Meiotic pairing, cross-overs and conversions are also unaffected by these changes in the centromere region. Therefore, at least for chromosomes III and XI, the centromeres are not chromosome specific. Based on the available sequence data for *CEN3*, *CEN11*⁵ and *CEN6*⁴, they may, in fact, all be structurally very similar. The essential region could consist of an extremely A+T-rich region (element II) whose exact sequence is unimportant, plus a relatively small flanking region (element III) whose sequence is highly conserved.

The issue of chromosome specificity of centromeres has also been examined in several other systems, and in detail in *Neurospora crassa* by D. D. Perkins, E. G. Barry and N. B. Raju (personal communication). These workers have intercrossed two reciprocal translocations whose break points are immediately to the right and to the left of centromeres III and V in order to study the effect of centromere substitutions in *Neurospora*. Their results are similar to ours, suggesting that meiotic pairing and behaviour of the centromere-substituted chromosomes is dependent on the homology between chromosome arms and is independent of which centromere the chromosomes possess. Also, Kasha has evidence in *Zea mays* that the centromere of one chromosome can be deleted and replaced by a centromere of a non-homologous chromosome without lowering plant fertility or interfering with functioning of the gametes¹⁴.

Finally, the ease with which alterations can be introduced into the centromere region of a chromosome *in vivo* and the sensitivity of the chromosome to proper centromere function make replacements or substitutions the method of choice for systematically investigating structure-function relationships in centromere DNAs.

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Identification and chemical synthesis of a tandemly repeated immunogenic region of *Plasmodium knowlesi* circumsporozoite protein

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Complementary DNA clones that code for the immunogenic region of the Plasmodium knowlesi circumsporozoite protein were shown to contain a tandemly repeating 36-base pair unit. A synthetic dodecapeptide corresponding to the predicted reading frame of the repeating nucleotide unit behaved, in an immunoradiometric assay, identically with the native P. knowlesi circumsporozoite protein. The repeating 36-base pair unit occurred 12 times within the gene and accounts for at least one-third of the amino acid sequence of the surface antigen protein.

THE sporozoite stage of the *Plasmodium* protozoan parasites that cause malaria are covered with a highly immunogenic surface protein, the circumsporozoite protein¹. In the mosquito salivary glands, approximately 10–20% of the protein synthesized by the sporozoite consists of this particular protein², which must have an important function. It may perhaps protect the parasite against the external environment, particularly against the defence mechanisms of its vertebrate host, and possibly participate in the process of invasion of the vertebrate host liver cells³. The structure of this protein could therefore be important in understanding the nature of the sporozoite cell surface, the molecular mechanism of the parasite interaction with liver cells and the nature of its antigenic determinants (epitopes). Knowledge of the latter would be significant in that it could lead to the chemical synthesis of peptides that may be used as a malaria vaccine. Unfortunately, however, the number of sporozoites that can be obtained from infected mosquitoes is very limited and as sporozoites cannot be cultivated *in vitro*, there is no source large enough to isolate and characterize the surface protein directly. For this reason, we have approached the problem by analysing the corresponding gene and recently have reported the cloning of cDNA fragments of *Plasmodium knowlesi* (H strain) surface antigen gene that contain the epitopes of the protein⁴.

We report here that the immunogenic region of the monkey malaria parasite *P. knowlesi* surface antigen consists of a 12 amino acid unit that is tandemly repeated 12 times and comprises at least one-third of the total amino acid sequence of the protein. A dodecapeptide corresponding to this unit has been synthesized and shown to have the properties of the *P. knowlesi* surface antigen epitope, as judged by its ability to bind monoclonal antibodies and to specifically compete in an immunoradiometric assay with the authentic *P. knowlesi* sporozoite surface antigen.

Screening of cDNA clones

The library containing the *P. knowlesi* surface antigen clones was constructed by inserting sized C-tailed cDNA made from infected mosquito thorax mRNA, into G-tailed *Pst*I-cleaved pBR322 (ref. 4). In this way, fragments of the *P. knowlesi* gene encoding the surface antigen epitope were expressed as a β -lactamase fusion protein, and were detected by an immune assay using anti-*P. knowlesi* monoclonal antibody. Three clones positive in the immunoradiometric assay were identified, and one of them (pEG81) contained only a very small DNA insert of approximately 350 base pairs (bp) as judged by restriction endonuclease mapping. Two other clones, pEG117 and pEG118, contained larger pieces of *Plasmodium* DNA, approximately 1,200 bp long. None of these clones, therefore, contained

the entire coding region of the gene which would be approximately 1,500 bp as estimated from a molecular weight of 51,000 for the protein not including the 3' and the 5' untranslated region.

These clones were shown in Southern and Northern blot analysis to be sporozoite-specific, cross-reacting with the sporozoite DNA and RNA, and with merozoite (blood form) DNA, but not merozoite RNA. In competition radioimmunoassays, the β -lactamase fusion proteins were shown to be specific for the *P. knowlesi* monoclonal antibodies⁴. The clones therefore must contain the portion of the *P. knowlesi* surface antigen gene which codes for the immunoreactive region of the circumsporozoite protein, and this must be contained within the 350-bp DNA insert of pEG81.

Nucleotide sequence of pEG81

To establish the nucleotide sequence of the small *Plasmodium* DNA fragment, it was first cleaved out of pEG81 with the restriction endonuclease *Pst*I and then re-inserted into the filamentous phage cloning/sequencing vector M13mp9 (ref. 5) at the *Pst*I cloning site in the β -galactosidase gene. M13mp9 clones containing the 350-bp insert in either orientation were then identified and sequenced using a synthetic universal primer and the Sanger dideoxy chain termination method^{6,7}. The *P. knowlesi* surface antigen gene fragment was found to consist

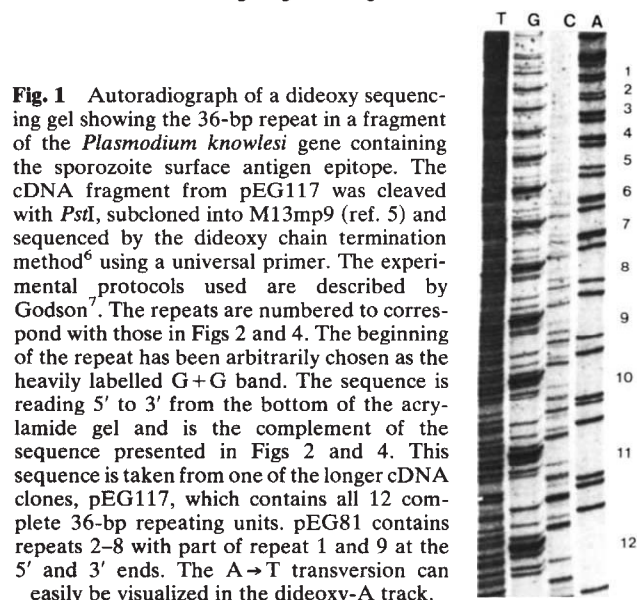
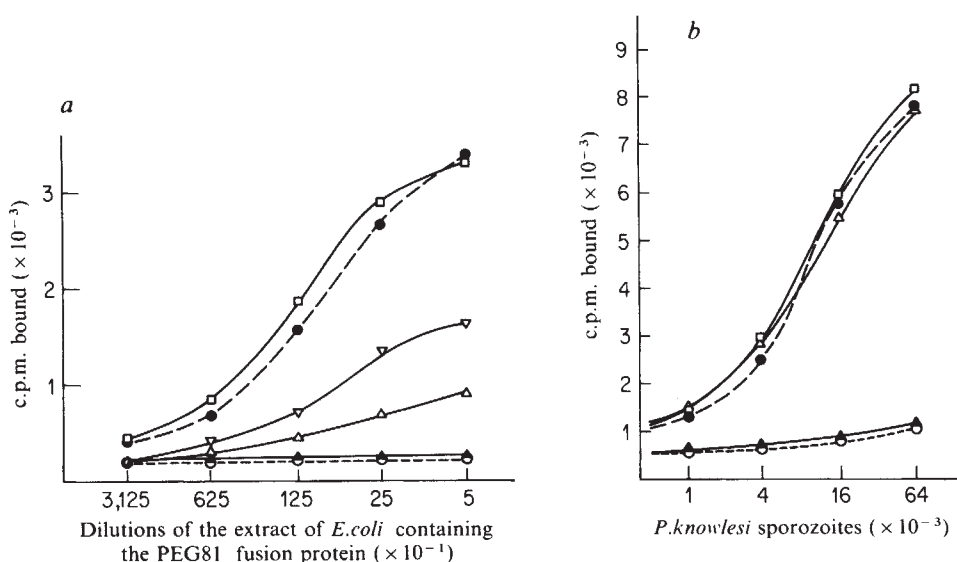


Fig. 1 Autoradiograph of a dideoxy sequencing gel showing the 36-bp repeat in a fragment of the *Plasmodium knowlesi* gene containing the sporozoite surface antigen epitope. The cDNA fragment from pEG117 was cleaved with *Pst*I, subcloned into M13mp9 (ref. 5) and sequenced by the dideoxy chain termination method⁶ using a universal primer. The experimental protocols used are described by Godson⁷. The repeats are numbered to correspond with those in Figs 2 and 4. The beginning of the repeat has been arbitrarily chosen as the heavily labelled G+G band. The sequence is reading 5' to 3' from the bottom of the acrylamide gel and is the complement of the sequence presented in Figs 2 and 4. This sequence is taken from one of the longer cDNA clones, pEG117, which contains all 12 complete 36-bp repeating units. pEG81 contains repeats 2–8 with part of repeat 1 and 9 at the 5' and 3' ends. The A→T transversion can easily be visualized in the dideoxy-A track.

Fig. 3 Treatment with proteolytic enzymes of the circumsporozoite surface protein of *P. knowlesi* and of partially purified ampicillin fusion protein containing repeated epitopes of the protein. **a** Shows the results of titration of the *E. coli* extracts containing the partially purified fusion protein, and **b** the titration of *P. knowlesi* sporozoite extracts. **a**, ●, Extract alone; □, plus trypsin (1 mg ml⁻¹); ▲, plus elastase (100 µg ml⁻¹); △, plus elastase (8 µg ml⁻¹); ▽, plus elastase (4 µg ml⁻¹); ○, control, PBS-BSA. **b**, ●, Extract alone; □, plus trypsin (1 mg ml⁻¹); ▲, plus elastase (10 µg ml⁻¹); △, plus elastase and elastase inhibitor.

Methods: *E. coli* expressing the fusion protein was grown to stationary phase. To 1.35 g of pelleted bacteria we added 4.5 ml of 0.01 M Tris-HCl pH 7.5 containing 0.01 M β -mercaptoethanol. The suspension was sonicated and centrifuged for 40 min at 10,000g. The supernatant was dialysed against 0.01 M Tris-HCl pH 8.6 containing 0.02 M NaCl and subjected to chromatography in a DEAE-Sephadex column equilibrated with the same buffer. Elution was performed with a linear gradient formed by adding 0.3 M NaCl to the starting buffer. The fusion protein eluted in fractions with conductivity at 0 °C between 5.6 and 9.0 as monitored by a two-site immunoradiometric assay performed with monoclonal antibodies¹². The fractions were concentrated by ultrafiltration in an Amicon unit (PM30 membrane) and subjected to chromatography in a precalibrated Sephadex G-200 column. The fusion protein eluted with an apparent molecular weight of 45,000. Aliquots of the partially purified protein and of extracts of *P. knowlesi* sporozoites prepared as described elsewhere¹² were simultaneously digested with TPCK-trypsin or porcine elastase (Worthington). Before treatment with trypsin the proteins were heated at 100 °C for 30 min, reduced with 0.1 M β -mercaptoethanol in the presence of 6 M urea, alkylated with 0.12 M iodoacetamide and filtered through a small Sephadex G-25 column equilibrated in PBS pH 7.5 to remove small molecular weight materials. After trypsin treatment (1 mg ml⁻¹) for 1 h at 37 °C, excess soybean trypsin inhibition was added. The digestion with elastase was performed in Tris buffer 0.1 M pH 8.6 at 37 °C for 30 min. Two samples of *P. knowlesi* extract were simultaneously treated with elastase. The synthetic elastase inhibitor, Ac-Ala-Ala-Pro-Ala-CH₂Cl (Enzyme Systems Products)¹¹ was added to one of them before incubation with the enzyme while in the other sample the inhibitor was added at the end of the incubation. The effect of the various treatments on the CS protein epitopes was evaluated by a two-site immunoradiometric assay¹². As shown, the reactivity of the antigens with the monoclonal antibody was not affected by reduction, alkylation and trypsin treatment following heating at 100 °C. In contrast, the reactivity of both antigens with the antibody was abolished by digestion with elastase.



tion terminators throughout the entire insert, and by interpolating 23 G residues between the known reading frame of the β -galactosidase gene⁹ and the repeated *Plasmodium* sequence, a reading frame of dodecapeptide subunits was predicted which started with Asp-Gly-Ala-, as shown in Fig. 3. This reading frame would be the same in pEG81 because the reading frames of the β -lactamase and β -galactosidase genes are the same relative to the *Pst*I cleavage site used to clone in each case (5' XCT GCA GXX 3'). This prediction was also confirmed by subcloning the fragment into the plasmid pUC9 (ref. 10) in the correct orientation as judged by restriction enzyme analysis and detecting a β -galactosidase fusion protein in the cell lysate that was positive in the immune assay (data not shown). In the predicted reading frame, the A $\rightarrow$ T transversion and G $\rightarrow$ A transition resulted in third-position changes in glycine (GGA $\rightarrow$ GGT) and glutamine (CAA $\rightarrow$ CAG) codons respectively, further suggesting that the chosen reading frame was correct.

The deduced reading frame of the repeated epitope is alanine-rich (Ala₃, Gly₃, Gln₃, Pro, Asp, Asn) and is devoid of lysine and arginine. If this deduction is correct, the natural epitope of the *in vivo*-synthesized sporozoite surface protein should be resistant to trypsin, but sensitive to porcine elastase, an enzyme known to cleave peptides at alanine residues¹¹. This should also be true for the *Escherichia coli*-synthesized β -lactamase fusion protein. These predictions were confirmed in the experiments illustrated in Fig. 3. The β -lactamase fusion protein containing the immunoreactive region was partially purified from extracts of *E. coli* by sequential chromatography on DEAE-Sephacel and Sephadex G-75. At the same time an extract of *P. knowlesi* sporozoites was prepared. Both extracts were boiled over water vapours for 10 min, reduced and alkylated with iodoacetamide in the presence of 6 M urea and subjected to the enzymatic digestion. As shown in Fig. 3, the epitope represented in the

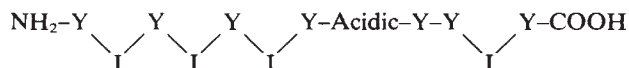
fusion protein and in the intact sporozoite protein have similar properties; that is, it resists boiling and trypsin treatment following complete reduction, but is destroyed in the presence of relatively low concentrations of elastase. The activity of elastase was totally blocked by the synthetic elastase inhibitor, Ac-Ala-Ala-Pro-Ala-CH₂Cl (ref. 11).

Chemical synthesis

To prove unequivocally that the deduced reading frame of the repeating nucleotide unit of the *P. knowlesi* surface antigen gene was correct, a dodecapeptide NH₂-Gln-Ala-Gln-Gly-Asp-Gly-Ala-Asn-Ala-Gly-Gln-Pro-CONH₂ and a tetraeicosapeptide (24 amino acids) containing the dodecapeptide repeated tandemly, were synthesized by and tested for reactivity in the standard two-site immunoradiometric assay that has been used throughout this work¹². The peptides were synthesized by solid phase synthesis^{13,14} using a benzhydrylamine resin. The finished peptide-resins were treated with hydrofluoric acid, to cleave the peptide from the resin support and to remove side-chain protecting groups. After purification by chromatography through a Sephadex G-25 column in the presence of 1 M acetic acid, the peptides were characterized and determined to be 97% homogeneous by amino acid composition and sequence analysis. To test for immunoreactivity the synthetic peptides were incubated in wells of microtitre plates pre-coated with 2G3, a monoclonal antibody raised against the CS protein of the H strain of *P. knowlesi*² and after washing, the wells were reincubated with ¹²⁵I-labelled 2G3 antibody and counted. As a control, the peptides were incubated in wells coated with a non-relevant antibody of the same isotype as 2G3. The results are shown in Table 2 and indicate that the tetraeicosapeptide but not the dodecapeptide (12 amino acids) can simultaneously bind two molecules of antibody, that is, the unlabelled 2G3 attached to

epitopes and that such an unusual structural feature is common to sporozoite surface antigens of all species of *Plasmodium*¹⁵. The findings described here therefore suggest that if equivalent peptides from the human malaras are immunogenic *in vivo*, they may be the basis of synthetic vaccines to the sporozoite stage.

The extraordinary, perfect conservation of the amino acid sequence of the repeated subunits suggests that this arrangement may be critical to the function of the parasite. The circumsporozoite protein not only presents surface recognition features to the external environment, that is, epitopes and cell receptor recognition sites, but could also function as a structural surface protein. The peptide subunit is rich in Gly, Ala and Glu and is made up of alternating hydrophilic (Gln, Asn) and hydrophobic (Gly, Ala, Pro) residues which give rise to a peptide of the following structure:



where I indicates hydrophilic and Y hydrophobic.

As this structure presents alternating hydrophilic and hydrophobic surfaces to the environment, it could be argued that this arrangement might provide for regular intermolecular packing, thus generating a tightly woven surface structure on the parasite membrane. This could provide a structural basis for the circumsporozoite protein in which sporozoites shed a morphologically distinct sheath after treatment with antibody to the CS protein. This hypothesis, however, would not explain the absence within the repeats of conservative amino acid substitutions which are found in other structural proteins displaying sequence periodicities, such as silk fibroin, zein, collagen, and resilin^{17,18}.

On the other hand, the sequence itself might be essential for some parasite function. For example, the dodecamer might be

a ligand for a membrane receptor on the host's cells, and consequently there would be strong selective pressures to maintain the correct amino acid sequence of the repeats. In this case, the presence of a regular array of a recurrent ligand would not only enhance the probability of an encounter between the parasite and the putative receptor, but also increase the chances that the interaction would be multivalent in nature and therefore much stronger. It is of interest that the streptococcal M protein type 24, a surface antigen, also contains a repeated peptide unit of 36 amino acids involved in protective immunity¹⁹. As with the *P. knowlesi* circumsporozoite protein, this repeating unit contains the epitope.

The presence of a repeated 36-bp sequence in the parasite DNA also raises the question of how it is not lost from the genome by recombination. In *E. coli*, repeated sequences are rapidly deleted from both plasmid and phage vectors unless the cells are *recA*⁻; in the *Plasmodium* cell the nucleotide repeats are presumably under similar pressures. Finally, the extraordinary conservation of the repeated nucleotide sequence and the alternation of two types of repeat, one with GGGT (unit 1, 3, 4, 6, 7, 10 and 11), the other with AGGA (unit 2, 5, 8, 9 and 12) (Fig. 4) suggest that this gene has evolved by intragenic duplication, but the precise mechanisms for such duplication remain to be determined.

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LETTERS TO NATURE

Outburst period-energy relations in cataclysmic novae

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SS Cygni is by far the best studied dwarf nova; since its discovery its brightness variations have been monitored almost continuously, mainly by members of the American Association of Variable Star Observers (AAVSO), the Variable Star Section of the British Astronomical Association and L'Association Française D'Observateurs D'Etoiles Variables (see Fig. 1). More than 600 outbursts have been observed to date. The eruption cycle of the dwarf nova SS Cygni behaves as a relaxation oscillator, with outburst energy correlated with the following

quiescent waiting period. Two distinct, wide and narrow outbursts exist. The outburst behaviour is closely related to outburst rise-time, and to the quiescent brightness level. There is a strong tendency for low-amplitude, slow rise-time outbursts to be clustered in periods associated with bright quiescent levels. We show here that the behaviour of SS Cygni is in close agreement with theoretical predictions of dynamically unstable states of the red star when it fills the Roche-lobe, and is in conflict with the assumptions of early disk instability models.

SS Cygni outbursts with recurrence times between ~20 and 100 days, rising from a quiescent brightness $V_{qu} \approx 11.8$ to a maximum $V_{max} \approx 8.5$. The steepness of the rise from quiescence to maximum ranges between ~2 and ~10 days. Based on this steepness of rise, Campbell¹ introduced an ordering of outbursts, which, in order of decreasing steepness are classified as A, B, C and D. Examples of each class are shown in Fig. 2. The most striking property is the constancy of the outburst decay-time independent of the steepness of rise in most eruptions (with exceptional long decays always associated with slow rises—classes C and D). This minimum value of the decay rate is an example² of the 'Bailey' relationship.

Sterne and Campbell³ have conducted an extensive statistical

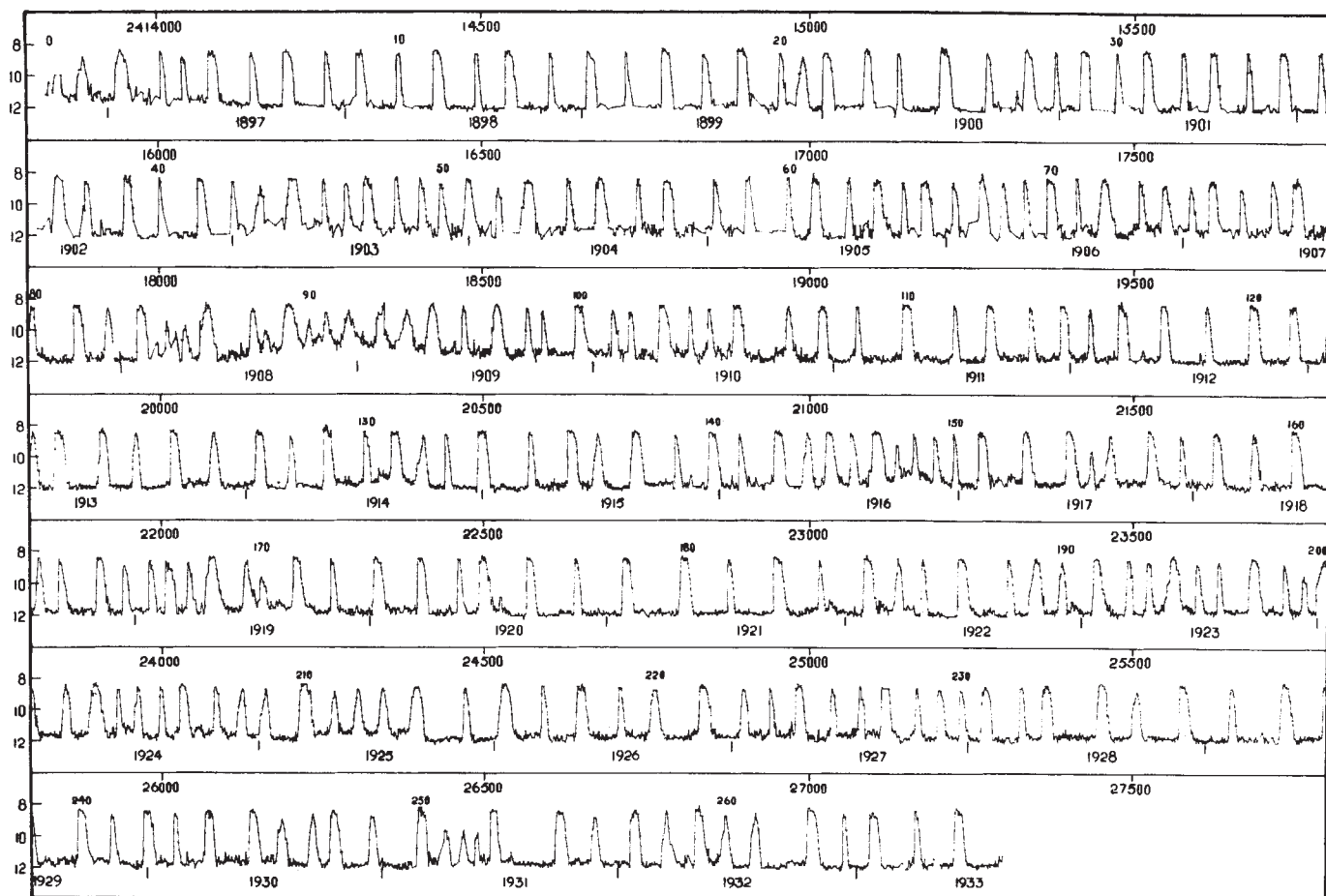


Fig. 1 Light curve covering the period 1897–1933. The data set analysed also includes the period 1940–79.

study of SS Cygni covering the period 1897–1933. We have extended their data set to include the years 1940–79^{4–7}. The main results of Sterne and Campbell are confirmed by our analysis, and can be summarized as follows:

(1) The frequency distribution of the width of the outbursts is bimodal, with maxima near ~ 7.5 and ~ 15 days, and a gap near ~ 11 days (Fig. 3). We define width as the time between visual magnitude 10.0 on the rising and declining branch of the outburst. The bimodal distribution leads to a natural distinction between narrow and wide outbursts. Because width is positively correlated with maximum brightness³ and because it varies over a substantial range (5–20 days) whereas $V_{\max}$ changes only by ~ 0.2 mag, it is a more sensitive energy indicator.

(2) There is a marked preference for narrow and wide outbursts to occur in an alternating pattern. Narrow outbursts are followed by wide with a frequency 1.46 times random expectation. Similarly wide–narrow patterns occur with a frequency 1.44. In contrast sequences narrow–narrow and wide–wide occur with frequencies 0.64 and 0.42 respectively.

(3) The width of an outburst (outburst energy) is correlated with the time interval to the next burst, as first shown by Kruytbosch⁸, but width is not correlated with the interval since the beginning of the previous burst (see Fig. 4). The correlation coefficients, for assumed linear relations, are 0.516 ± 0.031 and 0.089 ± 0.042 respectively. This compares with coefficients 0.486 ± 0.047 and 0.038 ± 0.047 derived by Sterne and Campbell³. Note that a similar correlation is found between width of outburst and width of following, but not preceding, quiescent state.

The properties of the outbursts can be analysed in more detail by examining classes A–D separately, when the following patterns emerge.

The average width of both the narrow and wide outbursts increases from types A to D, and the gap in the frequency

distribution moves from 11 days for class A to 14 for C and D classes. The increasing width is primarily due to the increased rise-time between A and D outbursts with a relatively constant decay-time.

In addition, amongst the C and D classes, there is a striking excess of very narrow outbursts (width < 5 days). These are outbursts which fail to reach a typical maximum brightness of ~ 8.5 mag by a wide margin (> 0.5 mag). If these exceptional cases are excluded then the width of narrow C and D outbursts is further enhanced, and the width–rise-time correlation amplified.

The burst width is correlated with the average visual maximum magnitude. Narrow outbursts average $V_{\max} \approx 8.63 \pm 0.28$ and wide outbursts $V_{\max} \approx 8.39 \pm 0.14$. With the removal of exceptional C and D outbursts this feature is shown by all four classes separately.

Figure 4a reveals that the relation between outburst width and the following-cycle length is not a simple linear relation. Narrow bursts can be followed by a quiescent time interval as short as 20 days, whereas the minimum waiting time after a wide burst is ≈ 35 days. Within each of the two groups the length of the following cycle has a broad statistical spread, with average waiting times of 43 ± 14 and 59 ± 12 days respectively. This scatter in the following-cycle duration is considerably larger than the scatter in the width, and within the narrow and wide groups individually there is a less striking correlation (see Fig. 4a).

The correlation between width and following-cycle time and the lack of correlation between width and preceding-cycle time applies separately to classes A–D. However, examination of the preceding-cycle time for individual classes yields a most striking result. The more irregular, slow rise-time, long decay-time, low-amplitude class D outbursts (both the narrow and the wide ones) have, on average, significantly shorter preceding-

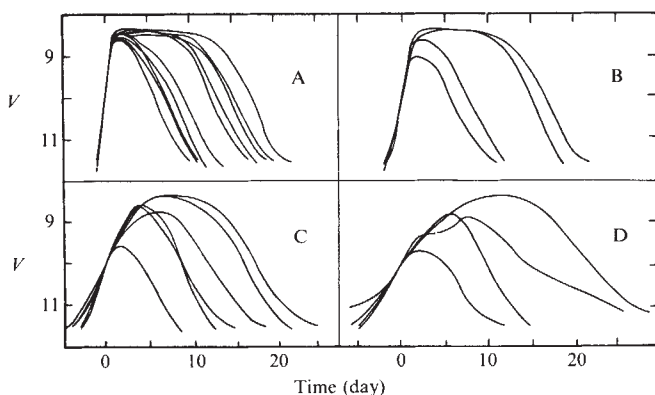


Fig. 2 Classification scheme of outbursts, due to Campbell, based on steepness of rise from quiescence to maximum. Note the approximately constant, minimum decay-time of classes A and B. Exceptional, extended decays are associated with slowly rising, lower-amplitude outbursts of classes C and D.

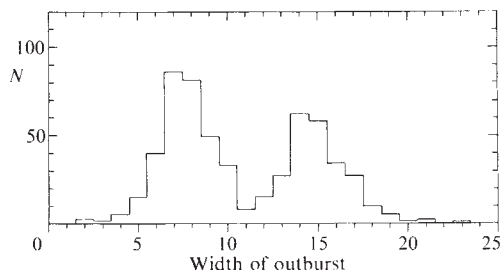


Fig. 3 Bimodal distribution of outburst widths.

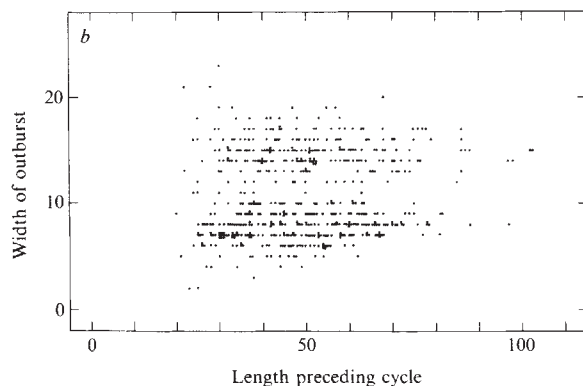
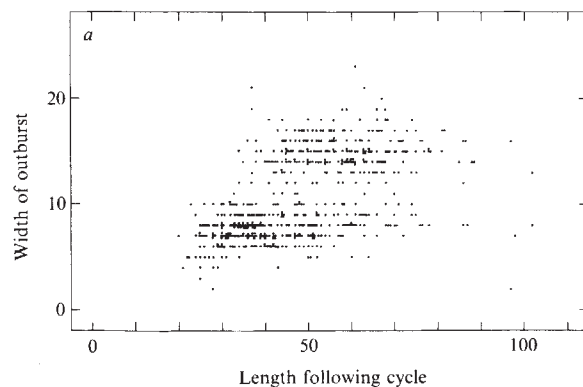


Fig. 4 Width of outburst versus outburst cycle-time of: *a*, the following quiescent period; *b*, the previous quiescent period.

cycle lengths; they tend to come 'too early'. This suggests that the D class outbursts are due to the system undergoing instability before it is fully relaxed, generating slowly-growing, low-amplitude (occasionally, very low-amplitude) eruptions which also slowly decay. As noted below, these are correlated with a brighter quiescent state.

When the distribution of classes A to D is examined we find that pairs of bursts of the same type tend to occur with greater frequency than a random distribution. Thus pairs of the same class occur together with an overall frequency 1.16 greater than random (and in particular, class D is grouped in pairs 4.50 times more frequently than random). One difference of speed class (AB, BD, CD) reduces the paired frequency to 0.99, two differences (AC, BD) to 0.82, and three differences (AD) to 0.47, compared with random. There is thus a strong tendency for the system to undergo a sequence of similar class outbursts. Large-amplitude, rapid-rise outbursts of classes A and B repeat in succession, as do slower-rise, lower-amplitude outbursts of classes C and D, with little mixing between extreme types.

This behaviour is closely related to a second feature which is statistically significant. The rise-time of the outburst (that is the speed class) is correlated with the quiescent magnitude between outbursts. Both the preceding quiescent brightness and the following quiescent brightness are correlated. Class A develop from faintest quiescent levels at $V = 11.90 \pm 0.12$ through increased brightness for classes B and C, to class D which outbursts from a level $V = 11.64 \pm 0.30$ (as no quiescent magnitudes are given in AAVSO reports our analysis is restricted to the period 1897–1933).

If we take outburst pairs with an intervening quiescent level $V < 11.7$ we find that in all 14 such cases a slow burst of either type C or D is involved, whereas only ≈ 6 are expected at random. In 10 cases periods of bright quiescence are preceded or followed by type D bursts, against the ≈ 1.5 expected by chance. Visual inspection of the 1940–79 light curves indicates

that this bunching, accompanied by increased quiescent brightness, is a characteristic property of the system.

We now examine the extent to which the outburst behaviour of SS Cygni is in agreement with red component instability models. Recombination of plasma in the H/He I and He II ionization zones can act dynamically to destabilize the envelope of the secondary red-component when its photosphere approaches close enough to the gravitational saddle point at the inner lagrangian point^{9,10}. Although the instability, acting along the line of centres of the binary system, takes place in a gravitational field which is not a $1/R$ potential, its basic features can be illustrated in such a single star potential. The main effect of the Roche potential is to confine the instability to a patch covering the lagrangian point with dimensions of the order of the vertical scale-height, z where

$$z \approx \left(\frac{\mathcal{R} \bar{T} R_2^3}{GM_2} \right)^{1/2} \quad (1)$$

In suitable binary configurations it is energetically possible for a region within a depth ΔR of the surface to be expelled across the saddle point, where ΔR is approximately given by

$$\Delta R \approx \frac{I R_2^2}{GM_2} \quad (2)$$

Here I is the ionization energy per gram, $I \approx 2 \times 10^{13}$ erg g⁻¹. The mass of the burst will be

$$\begin{aligned} \Delta M &\approx 2\pi \Delta R z^2 \bar{\rho} \\ &\approx 2\pi \bar{I} \bar{P} R_2 \left(\frac{R_2^2}{GM_2} \right)^2 \end{aligned} \quad (3)$$

where $\bar{\rho}$, $\bar{P}$ and $\bar{T}$ are the characteristic density, pressure and temperature of the unstable region.

Both linear adiabatic eigenvalues and fully developed hydro-

dynamic models have demonstrated the existence of such instabilities, though both their existence and properties are sensitive to the degrees of superadiabaticity in the ionization regions and hence to the assumed efficiency of convection. Stability studies have included convection, whilst models in ref. 10 ignore convection and favour instability. Accretion of the burst by the white dwarf through an $\alpha \approx 1$ disk will generate disk eruptions with the time-dependent photometric and spectroscopic properties observed (as discussed in detail in refs 11–13). The eruption energy is

$$E \approx \frac{GM_1 \Delta M}{R_1} \quad (4)$$

The cycle-time to the next outburst is given by the thermal time scale for relaxation of the envelope to thermal equilibrium. Following outburst the envelope collapses and only regains its expanded, thermal equilibrium state following energy transfer from the interior, after a time τ where

$$\tau \approx \frac{\mathcal{R} \bar{T} \Delta M}{\Delta L} \quad (5)$$

Here ΔM is the mass of the thermally depleted region. By conservation of mass this will, in a time-averaged sense, approximately equal the mass in the previous burst. ΔL is the luminosity deficit which must be restored in the outer envelope. In general this is a significant fraction of the luminosity of the star, but confined to a patch of dimensions $\approx 2\pi z^2$ on the surface. Thus

$$\Delta L \approx \frac{L_2 z^2}{2R_2^2} \quad (6)$$

and

$$\tau \approx \frac{2GM_2 \Delta M}{L_2 R_2} \quad (7)$$

Substituting from equation (4) we obtain an outburst energy-period relation for the following quiescent cycle of the form

$$\tau \approx 2 \left(\frac{M_2 R_1}{M_1 R_2} \right) \frac{E}{L_2} \quad (8)$$

We now examine the extent to which these relations satisfy the observed eruption properties of SS Cygni. The optical outburst energy of SS Cygni ranges from 2 to 8×10^{39} erg (ref. 14). To compute the total energy E the bolometric corrections must be estimated. Because most of the flux from the accretion disk is emitted in the UV–soft X-ray region, bolometric corrections are large. We take a factor ≈ 100 , based on the observed visible fraction in VW Hydri during decline¹⁵.

Spectroscopic masses indicate $M_1 \approx 1.33 M_\odot$ and $M_2 \approx 0.80 M_\odot$. We therefore take $R_1 = 5 \times 10^8$ cm and $R_2 = 0.93 (M_2/M_\odot) R_\odot = 5.1 \times 10^{10}$ cm assuming a main sequence secondary, for which $L_2 \approx (M_2/M_\odot)^{4.5} L_\odot = 1.4 \times 10^{33}$ erg. We obtain $\tau = 20$ –80 days, in agreement with the observed cycle-time. Mass-transfer instabilities thus provide a natural explanation of the observed relation.

The mass transferred in the burst can be estimated from equation (3). In main sequence stars, the ionization zones of H I and He I are at temperatures $\approx 15,000$ K and the He II zone at $\approx 30,000$ K, with corresponding pressures 10^6 and 10^8 dyn cm⁻². The expected masses involved are in the range 10^{22} – 10^{24} g, depending on the ionization zone responsible. The corresponding burst energies are 4×10^{39} – 10^{41} erg, or optical outburst energies 4×10^{37} – 10^{39} erg. With burst masses $\approx 10^{24}$ g lasting 7–15 days the average mass transfer rate during outburst is $\approx 10^{18}$ g s⁻¹, as required to power outbursts with luminosities 10^{35} erg s⁻¹. Clearly ionization zone destabilization is well able to power the outbursts.

The quiescent mass transfer mechanism in the thermally relaxing state is not established but may be the result of strong stellar winds above the photosphere. Chromospheric winds will be much enhanced above those of single main-sequence stars in the low-gravitational potential in the vicinity of the L_1 point—

more like those of extreme supergiants, but with strong activity confined to a region with dimensions of the order of a few scale heights around L_1 . Quiescent mass transfer rates of 10^{15} – 10^{16} g s⁻¹ are not unreasonable in such conditions. In circumstances in which dynamical instabilities drive a more powerful mass-flux than the wind, the background mass-loss will have little effect on the instability itself. The quiescent mass-transfer flux can be treated as a small perturbation to the underlying envelope structure, and there is no reason to believe that it will remove the cause of instability.

Periods of weaker activity, as measured by the bunching of slowly-developing low-amplitude outbursts, would be a natural consequence of enhanced red-component stability at times when the background stellar wind is strong, and the star's envelope most thermally disturbed by high surface mass-loss rates. The behaviour bears some resemblance to the standstills observed in Z Cam type dwarf novae, which can be attributed to high rates of steady mass-transfer causing stabilization of outburst behaviour by the red star and the system entering an 'extended eruption' state.

The extended rise and decay times of classes C and D can be naturally produced by mass-transfer variations. They would occur when mass-transfer changes take place on a longer time scale than the viscous accretion disk time scale, with the viscous time scale being associated with the minimum decay time scale of A and B outbursts¹³. Similarly the extended duration of wide outbursts would be caused by extended mass transfer, possibly due to the local instability at the lagrangian point being spread around the equatorial belt of the red component through non-synchronous rotation. Such a model has previously been suggested¹⁶ as being responsible for the wide supermaxima of SU UMa stars.

The relaxation-oscillator nature of SS Cygni's outburst cycle is a basic feature and must be accounted for by any successful model of dwarf novae. Although it probably does not, at this stage, conclusively rule out accretion-disk instabilities, the relaxation behaviour is a natural consequence of models in which the outbursts are caused by dynamical instabilities due to superadiabatic gradients in the secondary star's envelope, in the vicinity of the inner lagrangian point. The alternative disk model of Osaki¹⁷ (which instigated much of the recent work on disk instabilities) is certainly in conflict with the outburst period-energy relation in SS Cygni.

The range in waiting times after both narrow and wide bursts indicates that the relaxation process contains more structural detail than a simple oscillator with a fixed trigger level. However, the range of possible values for the variables in equation (5) suggests just how complex the true process may be. When three-dimensional structure and possible non-synchronous rotation of the secondary are considered the complexity is even more manifest. We propose that the destabilizing property of ionization zones within semi-detached binaries be referred to as the alpheus effect (see ref. 18).

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γ -Ray line emission from SS433

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We report here preliminary evidence for γ -ray line emission from the region of SS433. The observations were accomplished with the high-resolution γ -ray spectrometer aboard the HEAO 3 satellite¹. One of the line features reported here, located at an energy of 1.5 MeV, has a statistical significance of $\sim 6\sigma$ during a particular 18-day interval. Another feature appears near 1.2 MeV. Both features exhibit fractional linewidths, $\Delta E/E$, of $\sim 1\%$. The intensity of the 1.5-MeV feature is variable by a factor of ~ 3 on a time scale of days with a 46-day time average value of $1.5 \pm 0.3 \times 10^{-3}$ photons $\text{cm}^{-2} \text{s}^{-1}$. The 1.2-MeV feature is similarly variable with a time average intensity of $1.1 \pm 0.2 \times 10^{-3}$ photons $\text{cm}^{-2} \text{s}^{-1}$. The combined power in the lines is $\sim 2 \times 10^{37}$ erg s^{-1} , assuming isotropic emission from SS433. Phenomena previously observed^{2,3} from SS433 have been interpreted in terms of a kinematic model^{4,5} in which two oppositely directed jets of relativistic material ($v = 0.26c$) are ejected from a central source. Our observed energies may be explained in terms of this kinematic model as blue- and red-shifted components of the 1.369-MeV line from a nuclear transition of ^{24}Mg from its first excited state to its ground state. Both components appear to vary in energy over the duration of the observation in a manner roughly consistent with the pattern seen in the optical regime², in which 'movement' of optical features is accounted for by a precession of the axis of the jets with a 163.51-day period⁶. However, this identification implies an anomalously high magnesium abundance relative to carbon and oxygen.

The HEAO 3 observations were obtained from 10 October to 5 November 1979 and from 4 April to 25 April 1980, during which time SS433 was in the $\sim 30^\circ$ FWHM field of view of the germanium detectors. Although SS433 has been observed at energies up to ~ 30 keV (ref. 7) no previous observations in the low-energy γ -ray regime have been reported. Our search for line emission was based on the supposition that the putative ~ 33 MeV per nucleon jets should generate nuclear lines if they interact with ambient material. Accordingly, we studied spectra over the full range of detector energies ~ 50 keV to ~ 10 MeV.

The HEAO 3 satellite scanned a great circle in the sky once every 20 min. Spectra were accumulated over many scans, typically for 3 days. Net spectra from the region of SS433 were produced by selecting data within $\pm 15^\circ$ of SS433's scan angle position and subtracting from them data over a 78° wide control region. The spectra so obtained were searched by eye for any evidence of line emission.

A line which had a statistical significance of $2\text{--}4\sigma$ in each 3-day interval near 1.5 MeV was detected in several consecutive 3-day spectra. The superposition of six such consecutive background subtracted spectra, from 10.6 to 29 October 1979, is shown in Fig. 1. A feature at 1.497 MeV, consistent with a gaussian line shape with a width of ~ 8 keV FWHM, is seen. The statistical significance of this feature may be calculated by comparing the χ^2 values for the fit shown ($\chi^2 = 19.0$ for 20 d.f.) with the χ^2 value (62.8 for 23 d.f.) for a fit assuming no peak present. The likelihood ratio⁸ which gives the relative likelihood of the no-peak hypothesis to the peak hypothesis is, in this situation, approximately $\exp(-\Delta\chi^2/2)$. This number is 3×10^{-10} , equivalent to a 6.3σ detection. The high statistical significance of this detection must be reduced by the number of independent trials used in this search for emission from

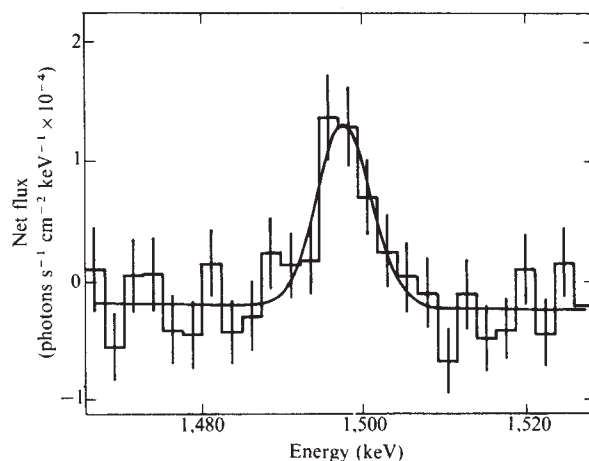


Fig. 1 Net γ -ray flux spectrum from the region of SS433 within ± 30 keV of the 1.5-MeV feature.

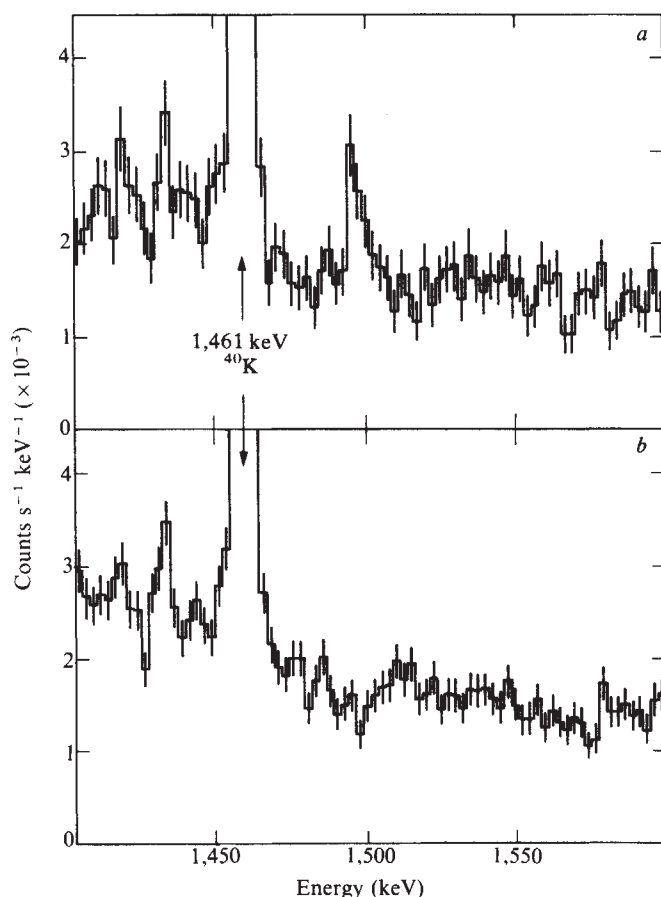


Fig. 2 Count rate spectra: a, from a 30° wide region centred on SS433; b, from a 78° wide nearby background region.

SS433. The number of independent energy bins is $\sim 2 \times 10^3$ and there is a further factor of ~ 10 associated with various line widths searched. Thus, the probability of a spurious detection must be increased to $\sim 10^{-5}$. This is still sufficiently small to give us confidence that a line feature has in fact been detected, and we take that point of view in what follows. Figure 2 shows counting rate spectra within ~ 100 keV of the 1.5-MeV feature from both the SS433 region and the background region. A strong background line at 1.46 MeV from the ^{40}K instrument calibration line is off-scale in both spectra, however there is no evidence of a background line in the vicinity of the 1.5-MeV feature.

A scan-angle plot of the events from 1,490 to 1,504 keV of Fig. 2a shows a peak consistent with the detector response

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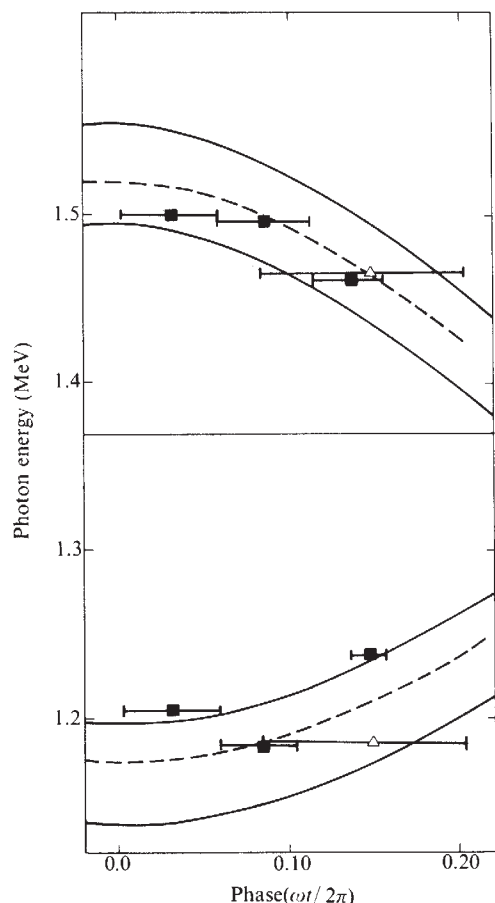


Fig. 3 Observations from autumn 1979 (■) and spring 1980 (△) as a function of the phase, $\omega t/2\pi$, of the kinematic model. The horizontal bars show the duration of each observation; the energy errors associated with each are given in Table 1. The curved lines are displaced ± 30 keV from the kinematic model calculations for $v = 0.26c$. The dashed line corresponds to $v = 0.25c$. Note that because the vertical axis is energy and not wavelength the figure is 'upside-down' compared with customary presentations of SS433 optical data.

function to a point source, located at a scan angle of $26 \pm 3^\circ$. The scan-angle location of SS433 is 27.2° . The nearest known low-energy γ -ray source to SS433 is Cygnus X-1, 27° away in scan angle. Therefore, on the basis of the positional agreement and the lack of other suitable candidates, we associate the line feature with SS433.

Ramaty *et al.*⁹ have compiled an extensive list of γ -ray lines expected from energetic particle interactions in astrophysical sites. None of these corresponds to the 1.5-MeV feature. The failure to detect the line in the 1,490–1,504-keV band after 29 October 1979 along with some evidence of line broadening beginning about 23 October suggested that the feature may be changing in a fashion similar to the well documented movement of the optical lines². Therefore, we have explored the possibility that the line is shifted in accordance with the parameters of the kinematic model. A recent fit⁶ to ~ 3 yr of optical data from SS433 to a sinusoidal Doppler variation gives $E_{\text{obs}}/E_{\text{rest}} = [1.0356(1 \pm 0.0863 \cos \omega t + 0.0467)]^{-1}$ with a period of 163.51 days. According to the fit, $\omega t(\text{mod } 2\pi) = 0$ on 10.37 October, right at the start of our autumn observations, producing a maximal red- or blueshift of magnitude 0.852 or 1.114.

From the tabulation of ref. 9, only two possible transitions could account for the feature: a blueshifted decay of the first excited state of ^{24}Mg at 1.369 MeV or a redshifted decay of the first excited state of ^{28}Si at 1.779 MeV. A more extensive examination of our observations selects the ^{24}Mg transition as the more likely for the following reasons. If on average the two jets are similar, then the red- and blueshifted intensities should

Table 1 History of SS433 line observations

Day (UT)	Line energy (keV)	FWHM (keV)	Intensity (10^{-3} photons $\text{cm}^{-2} \text{s}^{-1}$)
1.5-MeV line			
10.6–20.0			
October 1979	$1,500 \pm 3$	~ 7	0.68 ± 0.27
20.0–29.0			
October 1979	$1,497 \pm 3$	~ 7	1.97 ± 0.38
29.0 October–			
5.0 November 1979	$1,462 \pm 5$	~ 10	1.71 ± 0.53
4.5–25.0			
April 1980	$1,465 \pm 10$	< 30	1.53 ± 0.58
		Time average	1.47 ± 0.28
1.2-MeV line			
10.6–20.0			
October 1979	$1,205 \pm 5$	~ 12	1.45 ± 0.39
20.0–29.0			
October 1979	$1,185 \pm 5$	~ 12	1.20 ± 0.44
29.0 October–			
1.5 November 1979	$1,224 \pm 5$	~ 5	$0.69 \pm 0.56^*$
1.5–5.0			
November 1979	$1,237 \pm 5$	~ 5	2.23 ± 0.65
4.5–25.0			
April 1980	$1,185 \pm 10$	< 30	0.82 ± 0.28
		Time average	1.12 ± 0.18

* This value is not plotted in Fig. 2 because its statistical significance is $< 2\sigma$.

be similar, having a difference due to relativistic kinematics of at most a factor of 1.6 at $\omega t(\text{mod } 2\pi) = 0$ (with the blue being greater) for isotropic emission in the jet rest frame. We find evidence for a line feature which is consistent with redshifted ^{24}Mg decay but no feature which could be associated with blueshifted ^{28}Si decay. Furthermore, there is some evidence for a shift downward in energy for the 1.5-MeV transition as one moves to later times in the autumn, consistent with the pattern expected of a blueshifted line as one moves away from $\omega t = 0$. The evidence for a redshifted feature (in the vicinity of 1.2 MeV) is not as compelling as that of Fig. 1. The spectrum from 1.14 to 1.24 for the same 18-day interval as Fig. 1 does not show any strong feature. However, when the observations are divided into two consecutive 9-day intervals, a single line feature is seen in each half having statistical significances of 3.7 and 2.7 σ , with an apparent energy shift between the two data sets of ~ 20 keV.

Figure 3 shows a complete set of our observations in the energy range 1.13–1.54 MeV along with the band of 'predicted' energies based on the kinematic model as a function of $\omega t/2\pi$. The solid lines drawn in Fig. 3 are displaced ± 30 keV from the predictions of the simple model in accordance with variations of approximately this maximum amplitude seen in the optical regime⁶. If one pursues the identification of the line as blue- and redshifted radiation from ^{24}Mg de-excitation, then one simple model would have ^{24}Mg with the beam velocity ($v = 0.26c$) interacting with ambient protons at rest, giving rise to a velocity spread of $v = 0.24$ to $0.26c$ for the recoil $^{24}\text{Mg}^*$ for scattering angles of 0° – 180° . Therefore, the γ -ray emission would be from nuclei with a velocity diminished on average by $\sim 4\%$ from the beam velocity. The dashed line shown in Fig. 3 is calculated on this assumption, namely that the velocity of the emitting $^{24}\text{Mg}^*$ is $0.25c$.

The intensities associated with each of the measured points in Fig. 3 are given in Table 1. These intensities are derived from a better analysis than the simple superposition procedure which led to Fig. 1. This analysis guards better against errors due to changing background conditions. However, the energies and widths given in Table 1 should be regarded as preliminary.

The overall pattern of the autumn observations seems to support the interpretation of the 1.5-MeV line as a moving blueshifted component with less clear-cut behaviour for the

1.2-MeV line. The spring 1980 observations are less sensitive to energy variation and linewidth inasmuch as radiation damage¹⁰ had caused a degradation in the energy resolution at 1.5 MeV from ~3.2 keV FWHM initially to 15–20 keV in the spring of 1980. Variations in the intensity of each line by a factor of three are apparent from Table 1. Stronger variation is apparent on time scales of ~2 days. We have also searched for evidence of unshifted 1.369-MeV radiation at which energy there is an instrumental background line. Our 3σ upper limit is $\sim 1 \times 10^{-3}$ photons $\text{cm}^{-2} \text{s}^{-1}$.

If we assume that these lines represent isotropic emission from SS433 at the distance³ of 5.5 kpc, a γ -ray line luminosity, L_γ , of $2 \times 10^{37} \text{ erg s}^{-1}$ results. This luminosity is a factor of 250 greater than the 2–10-keV X-ray luminosity⁷, and may be a significant fraction of the beam kinetic energy flow. Theoretical estimates of this quantity range from $\sim 10^{38}$ (ref. 11) to $\sim 10^{41}$ (ref. 12) erg s^{-1} . If the ^{24}Mg line identification is correct and

the line is collisionally excited, then the kinetic energy flow rate of ~800-MeV magnesium nuclei is $\geq 10^{42} \text{ erg s}^{-1}$ if the fraction of nuclei which contribute a 1.369-MeV γ ray is $\leq 10^{-2}$ as might be expected. This energy flow rate in magnesium is a very large number in view of the theoretical estimates. Finally, we have performed a preliminary examination of our data for other shifted nuclear lines and find none at comparable intensities. On the basis of solar abundances relative to ^{24}Mg and excitation total cross-sections at 33 MeV per nucleon, we would expect very strong lines from ^{12}C and ^{16}O which are 6–7 times the intensity of the apparent ^{24}Mg line. The absence of ^{12}C and ^{16}O and the apparent presence of ^{24}Mg place important constraints on future models of SS433.

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Limits on the Sun's core magnetism from solar oscillations

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Many years ago Cowling¹ discussed the possibility that the Sun has a significant relic field. This field would have poloidal and toroidal components, with the toroidal component being driven by dynamo action on the poloidal component. The toroidal field would be quadrupole in nature having opposite senses in the upper and lower hemispheres. Subsequently, Dicke² proposed that the solar quadrupole moment is caused by a strong, inclined toroidal field with a magnitude of $\sim 6 \times 10^7 \text{ G}$. Ulrich and Rhodes³ suggested that a poloidal field with a magnitude of $3 \times 10^8 \text{ G}$ was required to account for some of the properties of the 5-min period oscillation. Whereas Mestel and Moss⁴ claimed that such fields may not be sufficiently stable to endure. Hill *et al.*⁵ argued that solar oscillation data imply that a simple poloidal field is much weaker than $3 \times 10^8 \text{ G}$ and Gough⁶ has suggested that the toroidal field may be much weaker than the $6 \times 10^7 \text{ G}$ postulated by Dicke². Magnetic fields, like rotation, produce a fine structure in solar oscillations. Their effects should be detectable provided the fields are sufficiently intense. Here we perform an analysis of oscillation data due to Hill *et al.*⁵ to show that limits of a few megagauss can be placed on poloidal and toroidal magnetic fields inside the Sun. A limit can thereby also be placed on the part of the quadrupole moment of the Sun due to magnetism. These fields are too weak to induce a quadrupole moment much larger than that which would result if the Sun rotated rigidly at the observed surface equatorial rate.

To determine the fine structure of solar oscillations, the equation

$$\rho \frac{dv}{dt} = -\bar{\nabla} p + \frac{1}{4\pi} (\bar{\nabla} \times \bar{B}) \times \bar{B} + \rho \bar{g} \quad (1)$$

must be perturbed and solved. After perturbation (following

ref. 7), the magnetic field term in equation (1) becomes

$$\frac{1}{4\pi} \left\{ (\bar{\nabla} \times \bar{B}) \times \left[\bar{\nabla} \times \left(\delta \bar{r}_{nlm} \times \bar{B} \right) \right] + \left[\bar{\nabla} \times \left(\bar{\nabla} \times [\delta \bar{r}_{nlm} \times \bar{B}] \right) \times \bar{B} \right] \right\} \quad (2)$$

The normal coordinate of the oscillation, resulting from solution of the perturbed equations, is represented by

$$\delta \bar{r}_{nlm} = \left(\delta r, \delta r_\theta \frac{\partial}{\partial \theta}, \delta r_\phi \frac{1}{\sin \theta} \frac{\partial}{\partial \phi} \right) Y_l^m(\theta, \phi) e^{i\omega_{nl} t} \quad (3)$$

where n is the number of radial nodes in the oscillation and l its degree. After some manipulation, the frequency splitting due to magnetic field effects, ω_{nlm}^B , can be written as

$$\omega_{nlm}^B = \frac{1}{2\omega_{nl}} \sum_{i,j} \langle B_i T_{ij} B_j - \frac{1}{2} \delta \bar{r} \cdot \bar{\nabla} (B_i B_j) X_{ij} \rangle \quad (4)$$

The quantity inside the brackets represents the expectation value of that quantity with respect to the radial component of the normal mode, δr . This quantity is the manipulated form of the magnetic field term in equation (2) and ω_{nl} is the normal mode frequency in the absence of B . T_{nj} and X_{nj} are the components of 3×3 matrices in the normal modes and powers of their derivatives; the indices n and j represent r , θ , and ϕ and are summed over. After selecting a functional form for B and evaluating equation (4), we obtain the second-order correction due to the magnetic field of the frequencies of the normal modes. In particular, magnetic fields induce a non-uniform spacing of the $(2l+1)$ components of m for an (nl) multiplet.

Discussions of solar magnetism generally assume a relic poloidal field. Shearing of a poloidal field by differential rotation induces a toroidal field. Therefore, in the evaluation of equation (4), both poloidal and toroidal field effects are included. The poloidal and toroidal fields contribute separately to the splitting of normal modes of oscillation. Cross terms are imaginary and do not have any net effect on the magnetic equilibrium configuration considered here. These cross terms cause damping or driving; their effects will be discussed elsewhere⁸ (W. D., in preparation).

The most general multipole expansion of the field which satisfies $\text{div } B = 0$ and includes poloidal and toroidal components is, in polar coordinates:

$$\bar{B}(r, \theta, \phi) = \frac{1}{r} \sum_l \left[l(l+1) \frac{\chi_l(r) P_l}{r} + \frac{d\chi_l}{dr} \frac{dP_l}{d\theta} + \psi_l(r) \frac{dP_l}{d\theta} \right] \quad (5)$$

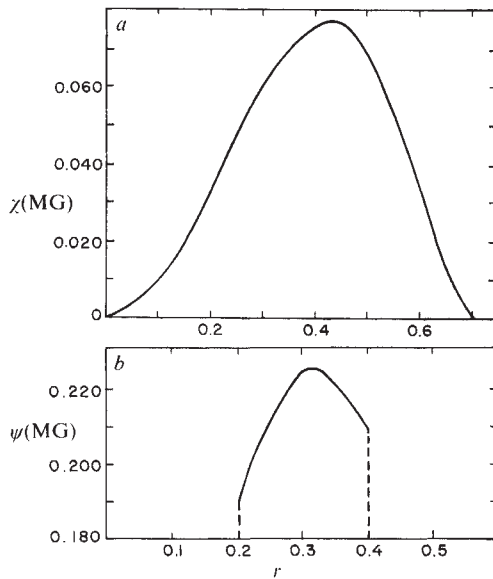


Fig. 1 *a*, The functional form of $\chi(r)$ defined in equation (7). *b*, The form of $\psi(r)$ from equation (10).

P_l is the Legendre polynomial of degree l in the multipole expansion and $\chi_l(r)$ and $\psi_l(r)$ are the magnetic strength functions. The $l=1$ term in the expansion is the poloidal term, which can be written as

$$\vec{B}_p(r, \theta) = \left[\frac{2\chi(r)}{r^2} \cos \theta, -\frac{1}{r} \frac{d\chi}{dr} \sin \theta, 0 \right] \quad (6)$$

The function χ is defined by

$$\chi(r) = \frac{r^2 B_c}{2} \left[1 - \left(\frac{r}{r_c} \right)^2 \right]^N \quad \text{if } r \leq r_c \quad (7)$$

and

$$\chi(r) = 0 \quad \text{if } r > r_c$$

For this dipole field, B_c is the maximum field value, r_c is the distance from the centre of the Sun to the edge of the radiative core, and N is taken to be 2, 10, and 50 in three different calculations of magnetic field effects. For these three values of N , the maximum value of B_p occurs at a fractional radius of 0.42, 0.22, and 0.10, respectively. This allows us to study the effect of different magnetic field limits. The function χ vanishes at the origin and at the edge of the radiative core. The field is assumed to vanish in the convection zone due to turbulence.

The $l=2$ term in the expansion yields the toroidal term. It is assumed to be induced by the poloidal field; the functional form of the $\hat{\phi}$ -component of equation (6) is chosen to match this condition. Generation of the toroidal component follows from Maxwell's equations:

$$-\frac{\partial \vec{B}_T}{\partial t} = \hat{\phi} r \sin \theta \vec{B}_p \cdot \vec{\nabla} \Omega(r, \theta) \quad (8)$$

The form of the toroidal field follows from equation (5):

$$\vec{B}_T = (0, 0, \psi \sin \theta \cos \theta) \quad (9)$$

Where ψ reflects its origin in differential rotation (equation (8))

$$\psi = \frac{r B_c}{\Omega_c} \frac{d\Omega}{dr} \left[1 - \left(\frac{r}{r_c} \right)^2 \right]^N \quad \text{if } r \leq r_c \quad (10)$$

and

$$\psi = 0 \quad \text{if } r > r_c$$

Here, Ω_c is the central angular velocity. This expression for the toroidal component reflects its origin in differential rotation. The field vanishes both at the origin and at the edge of the radiative core, and has opposite senses in the upper and lower hemispheres. For $N=2, 10$, and 50 , the maximum value of the

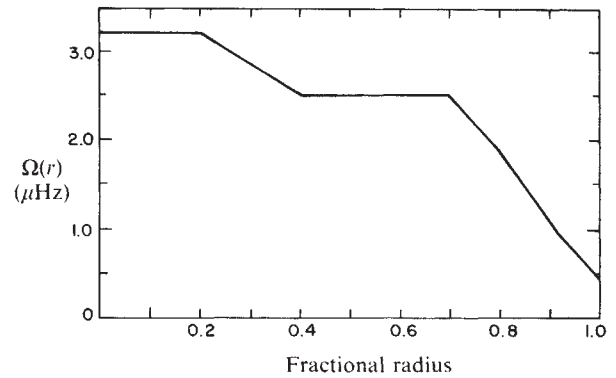


Fig. 2 The functional form of Ω used by Hill *et al.*⁵.

toroidal field occurs at a fractional radius of 0.32, 0.16, and 0.07, respectively. The functional forms of $\chi(r)$ and $\psi(r)$ are shown in Fig. 1*a* and *b* for $N=2$, $B_c=1$ MG and $r_c=0.72$. For the toroidal field, the functional form of Ω is that used by Hill *et al.*⁵ who assumed $\Omega = \Omega(r)$. A plot of Ω versus fractional radius is shown in Fig. 2. The poloidal and toroidal components are treated separately in calculating the magnetically-induced fine structure.

If the Sun can be treated as a slow rotator and magnetic field effects can be ignored, then the fine structure of solar oscillations would be expected to exhibit uniform spacings for the rotationally split elements of each (nl) multiplet. The non-uniformities in the fine structure of solar oscillation are attributed here to the presence of solar magnetic fields. These residuals are used, together with the field model given above, to obtain upper bounds to the component field intensities.

These upper limits can subsequently be used to define the driving term in Poisson equations in which the density perturbation is not derived from a potential. Solutions to these equations give the upper limits on the field's contributions to the Sun's quadrupole moment. The magnetic fields modelled here can be rotated by a simple coordinate transformation if a comparison with inclined fields, such as that proposed by Dicke², is desired.

The solar model used in our calculations is due to Dziembowski and Pamjatnykh⁸. It is a standard model of an evolved Sun, with initial and present fractional central hydrogen contents of 0.732 and 0.332, and an initial heavy metal abundance of 0.02. The depth of the convection zone is 0.28 of the solar radius.

The poloidal and toroidal magnetic fields under consideration here are confined below the convection zone. Consequently, we are interested in non-uniformities in the fine structure of the g modes, which sample the deep interior of the Sun. The first-order (Coriolis) effects of rotation yield a uniform fine structure with the individual g modes split by $\sim 3 \mu\text{Hz}$. We ignore second-order (centrifugal) effects so we ascribe the observed non-uniformities to magnetic field effects. The observed g modes used in the current analysis are presented in Table 1. Note that Dziembowski and Goode (in preparation) have shown that centrifugal effects are indeed negligible for these modes.

For a particular observed (nl) multiplet, the difference between the $m=l$ and $m=l-2$ states less the difference between the $m=-l+2$ and $-l$ states, as identified in ref. 5, is set to the degree of non-linearity, that is, the maximum non-uniformity between peaks in ref. 5 ($0.07 \mu\text{Hz}$). We note that the errors given in ref. 5 reflect the peak widths in the data. For each multiplet, the maximum non-uniformity combined with the results of equation (4), can be used to place an upper limit on the intensity of the magnetic field. Specifically, the change in frequency of an (nl) multiplet due to magnetic effects is

$$f_{nlm} = A_{nl}^p B_p^2 m^2 + A_{nl}^T B_T^2 m^2 + A_{nl}^T B_T^2 m^4 \quad (11)$$

where B_p and B_T (in megagauss) are the values of B_c from

Table 1 Observed splittings and calculated splitting coefficients (in μHz)

Mode	Mean splitting	N	Poloidal A_{nl}^P	Toroidal $A_{nl}^{T_2}$	Toroidal $A_{nl}^{T_4}$
$g_7, l=6$	2.902	2	-0.13×10^{-2}	0.15×10^{-3}	-0.32×10^{-5}
		10	-0.96×10^{-3}	0.38×10^{-4}	-0.74×10^{-6}
		50	-0.47×10^{-3}	0.38×10^{-5}	-0.70×10^{-7}
$g_9, l=8$	2.934	2	-0.12×10^{-2}	0.12×10^{-3}	-0.16×10^{-5}
		10	-0.97×10^{-3}	0.30×10^{-4}	-0.36×10^{-6}
		50	-0.49×10^{-3}	0.83×10^{-5}	-0.80×10^{-7}
$f, l=6$	2.920	2	-0.27×10^{-3}	0.10×10^{-4}	-0.25×10^{-6}
		10	-0.13×10^{-3}	0.56×10^{-5}	-0.12×10^{-6}
		50	$+0.11 \times 10^{-4}$	0.14×10^{-5}	-0.26×10^{-7}

Table 2 Maximum poloidal field (MG) and J_2^P

	$N=2$	$N=10$	$N=50$
B_P	1.0	1.1	1.6
J_2^P	0.2×10^{-6}	0.1×10^{-7}	0.1×10^{-8}

equations (7) and (10), respectively. The A s are the calculated coefficients presented in Table 1. The frequency changes for all three components in equation (11) depend on even powers of m . This occurs because the perturbation is quadratic in B and, therefore, independent of its sign. The m^4 term arises because the toroidal field has tensor rank two, allowing a hexadecapole distortion of the Sun.

Using the $g_9, l=8$ state, we ascribe the maximum of the observed non-uniformity to the poloidal field and obtain, for $N=2$;

$$0.07 = 8(l-1)B_P^2 A^P = 56(0.12 \times 10^{-2})B_P^2 \quad (12)$$

Thus, the upper limit of the poloidal field, for this case, is:

$$B_P \approx 1 \text{ MG} \quad (13)$$

This limit on the $N=2$ poloidal magnetic field component allows a corresponding limit to be placed on its contribution to J_2 :

$$J_2^P \leq 0.2 B_P^2 \times 10^{-6} \quad (14)$$

The corresponding values of B_P and J_2^P for $N=10$ and 50 are given in Table 2. As N increases, A_{nl}^P decreases because the field falls off more rapidly with increasing r as N increases. As N increases, J_2^P also decreases, but even more rapidly than A_{nl}^P due to its greater sensitivity to the field at larger values of r . In all three cases, J_2^P is comparable with or less than the dynamic J_2 which would result if the Sun rotated rigidly at its observed equatorial surface rate. Using the solar model of ref. 8, this value is 0.3×10^{-6} . In addition, for all three poloidal field cases, the quadrupole moment is oblate. This can be understood schematically by considering the resemblance of the poloidal field to that due to a current loop. The resulting Lorentz force on this imaginary current loop is directed radially outward. This, in turn, implies an oblate distortion of the mass with respect to the field's axis of symmetry.

Ascribing all of the non-uniformity to the m^2 component of the toroidal field, which has a distinctly different signature than the m^4 component, the $N=2$ equation for the $g_9, l=8$ mode is

$$0.07 = 8(l-1)B_{T_1}^2 A^{T_1} = 56(0.12 \times 10^{-3})B_{T_1}^2 \quad (15)$$

This yields the limit:

$$B_{T_1} \approx 3 \text{ MG} \quad (16)$$

The implied limit for J_2 is given by

$$J_2^{T_1} \leq 0.5 B_{T_1}^2 \times 10^{-8} \quad (17)$$

For $N=10$ and 50, we have

$$J_2^{T_1} \leq -0.7 B_{T_1}^2 \times 10^{-8} \leq 0$$

and

$$J_2^{T_1} \leq -0.3 B_{T_1}^2 \times 10^{-8} \leq 0 \quad (18)$$

respectively. The distortion is oblate for $N=2$ and prolate for the other cases. This can be understood by a current loop analogy. In the analogy, the distorting field is in the ϕ -direction in the upper hemisphere and in the opposite direction in the lower hemisphere. The radial distortion is caused by the θ component of the current loops ($\theta \times \phi = \hat{r}$). The current loops may be envisioned in cross-section as four distinct loops—each starts at a pole and runs along a longitude to the equator, then along an equatorial radius and along the polar axis to close the loop. The direction of the current depends on how rapidly the field falls off as a function of r . We find that small values of N yield an oblate distortion, while larger values of N give a prolate distortion of the mass. The coefficient of the toroidal field, as illustrated in equation (18), falls off for increasing N after J_2^T becomes prolate. For all three cases, the limit on the magnitude of J_2^T is comparable to that for the poloidal case.

Attributing all of the non-uniformity to the m^4 component of the toroidal field, we obtain, for the $g_9, l=8$ mode and $N=2$:

$$B_{T_2} \approx 3 \text{ MG}$$

and

$$J_4^{T_2} \geq -0.8 B_{T_2}^2 \times 10^{-9} \leq 0 \quad (19)$$

This implies a lower limit of -0.7×10^{-8} on the hexadecapole distortion for the functional form of ψ with $N=2$. For $N=10$ and 50 the values of J_4 are comparable. The origin of the prolate J_4 values can be understood with the same analogy that accounted for the toroidal field J_2 values, except that the θ component of the current loop always has one sign and the behaviour for small N is more complicated.

The symmetry properties of the global oscillation data of Hill *et al.*⁵ imply that the Sun has a single axis of rotation. This is, in part, the reason that the poloidal field chosen here is directed along the rotation axis. The toroidal field is in the ϕ -direction in the upper hemisphere and in the opposite direction in the lower hemisphere. Rotation matrices can be applied to the field vectors to incline the field and then new splittings calculated straightforwardly from the old ones. Dicke⁹ has calculated the splittings of some 5-min period oscillations due to an intense, inclined, rotating magnetic field. If the magnetic field effects, for these g modes, are calculated using Dicke's field, as given in ref. 10, the predicted spacings are strongly non-uniform. Approximating his field, in its rest frame, by

$$\psi = r^2 B_c \left[1 - \left(\frac{r}{r_0} \right)^2 \right]^2 \quad (20)$$

where $r_0 = 0.33$ and $B_c = 60 \text{ MG}$, we subsequently find a value of J_2 which is comparable to his. We also find that

$$A^{T_1} B_{T_1}^2 = 1.2 \mu\text{Hz}$$

for the $g_9, l=8$ mode. This value is about 1,000 times the value implied by the degree of spacing uniformity in the g -mode oscillation data. Thus, a strong toroidal field is not consistent with the approximately uniform spacing of the modes as observed and presented in ref. 5. Moreover, the inclined field, as postulated by Dicke², should result in a much denser oscillation spectrum than the one which is observed.

We have assumed various descriptions for poloidal and toroidal field components assumed to be trapped in the radiative core of the Sun. The radius at which a particular component has its maximum value varies with its description as does the

Table 3 Maximum toroidal field (MG), J_2^T , and J_4^T

	$N=2$	$N=10$	$N=50$
$m=2$			
B_{T_1}	3	6	12
J_2^T	$+0.5 \times 10^{-7}$	-0.3×10^{-6}	-0.4×10^{-6}
$m=4$			
B_{T_2}	3	4	12
J_4^T	-0.7×10^{-8}	-0.6×10^{-8}	-0.3×10^{-8}

value of the maximum. The observed degree of non-uniformity in rotationally split g modes is used to place an upper limit on the calculated magnetic fields and the concomitant quadrupole and hexadecapole distortions of the Sun. We conclude that magnetic fields in the deep solar interior do not induce a mass distortion comparable with that due to the rotating core shown in Fig. 2.

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Permian alkaline undersaturated and carbonatite province, and rifting along the West African craton

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Recent work^{1,2} carried out in north-east Mali, which showed the Pan-African orogeny (600 Myr) to be the result of a collision between the passive margin of the West African craton and the active margin of a continental mass situated to the east, has also outlined an entire province of alkaline undersaturated ring complexes and carbonatites, the first to be discovered in West Africa excluding Morocco^{3,4} (Fig. 1). This province is quite distinct petrologically and structurally from the Iforas alkaline younger granite province situated in the Pan-African domain east of the suture which has yielded Rb/Sr whole-rock ages of between 560 and 520 Myr (ref. 5). The undersaturated province is located along a well-defined rift on the edge of the West African craton which is totally devoid of Pan-African magmatism. We report here that the Permian radiometric age obtained for one of these intrusions shows the existence of a hitherto unsuspected important tectono-magmatic event in this part of Africa, which may have far-reaching structural implications as well as economic interest.

The alkaline undersaturated province largely masked by Cretaceous–Tertiary sediments comprises: a few typical ring-complexes including the Tadhak with diameter of 12 km (long. 0°, lat. 20° 30' N), Tikarkas 4 km in diameter (long. 0° 06' E, lat. 20° 44' E) and other occurrences about 50 km to the north-east which have not yet been studied⁶, several carbonatite plugs (long. 0° 06' E, lat. 20° 09' N, long. 0° 03' E, lat. 20° 04' N) which at present are being investigated by the United Nations Revolv-

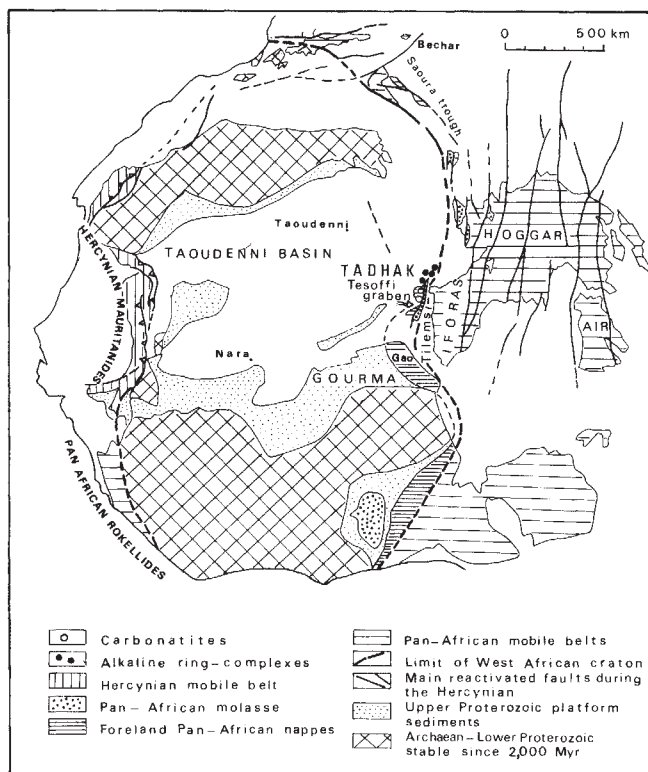


Fig. 1 North-east Mali showing the limit of the West African craton.

ing Fund, and NNE to NE trending dyke swarms displaying a wide variety of undersaturated rocks including microsyenites, phonolites and banded carbonatites. The Tadhak complex which has been dated is composed of four main units which are concentrically disposed: (1) an outer fine-grained nepheline syenite displaying pronounced flow structure; (2) a leucocratic nepheline syenite; (3) a lenticular unit of nepheline melteigite grading to a nepheline ijolite, containing enclaves of pyroxenite displaying cumulate textures; (4) an inner foyaïtic nepheline syenite. These phases are cut by numerous foyaïte and tinguaité dykes and a small carbonatite plug occurs in the eastern part of the complex⁴.

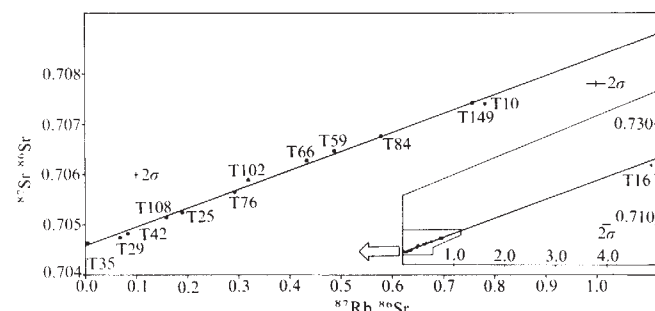
Thirteen Rb/Sr analyses of the Tadhak complex taken from the different parts of this composite intrusion (Fig. 2, Table 1) give a lower Permian age of 262 ± 7 Myr (MSWD = 2.7) with an $^{87}\text{Sr}/^{86}\text{Sr}$ initial ratio of 0.70457 ± 0.00004 (with the omission of sample T16: 12WR, 269 ± 9 , 0.70455 ± 0.00004 , MSWD = 2.2). The age and margin of error at 2σ level were calculated by the Williamson method⁷. In view of the absence of later deformation, this age is interpreted as dating the intrusion and the low initial ratio suggests a mainly mantle source.

The Tadhak and related alkaline plutons outcrop at the northern end of the Tilemsi trough which approximately coincides with the Pan-African suture. The suture is characterized by the presence of important positive gravity anomalies shown to be produced by unrooted metabasic and ultrabasic bodies⁸ thought to represent the relics of deep seated portions of island arcs⁹. Geological³ and geophysical¹⁰ data show that the Tilemsi trough, filled with Cretaceous to Eocene sediments (800 m), is partly superimposed on an earlier graben (Tesoffi graben, Fig. 1) filled with red molasse sediments which outcrops in a 10 km wide north-northeasterly trending strip 150 km long. The older graben contains over 2,000 m of purple arkosic sandstones and conglomerates and by analogy with the Serie Pourprée of northwestern Hoggar it was mapped as a Pan-African molasse. In the absence of palaeontological data, however, doubt may be cast on this attribution as the source of the sediments is from the west. This is indicated by the widespread occurrence of abundant pebbles of undeformed

Table 1 Rb/Sr analyses of samples from Tadhak

Samples	Rb (p.p.m.)	Sr (p.p.m.)	$^{87}\text{Sr}/^{86}\text{Sr}$	$^{87}\text{Rb}/^{86}\text{Sr}$
T10	139	514	0.70739 ± 4	0.7825
T16	154	91.4	0.72191 ± 5	4.882
T25	140	2140	0.70525 ± 5	0.1893
T29	98	4136	0.70474 ± 4	0.0688
T35	0.8*	1415	0.70463 ± 5	0.00164
T42	40.5	1398	0.70483 ± 3	0.0838
T59	221	1315	0.70647 ± 5	0.4862
T66	156	1039	0.70629 ± 4	0.4344
T76	546	5324	0.70566 ± 4	0.2967
T84	274	1368	0.70677 ± 4	0.3795
T102	160	1454	0.70591 ± 5	0.3184
T108	129	2345	0.70517 ± 6	0.1591
T149	179	683	0.70741 ± 4	0.7583

The isotopic composition and the concentration analyses were carried out at the Belgian Centre for Geochronology (MRAC-ULB). Rb and Sr concentration was measured by X-ray fluorescence (except* which was done by isotope dilution). Errors on Rb/Sr and $^{87}\text{Rb}/^{86}\text{Sr}$ ratios are 2%. Errors on $^{87}\text{Sr}/^{86}\text{Sr}$ are given at the 2σ level in 10^{-5} . 20 measurements on standard NBS987 give: 0.710218 ± 0.000035 . Normalization for $^{86}\text{Sr}/^{88}\text{Sr} = 0.1194$. $\lambda \text{Rb} = 1.42 \cdot 10^{-11} \text{ yr}^{-1}$.

**Fig. 2** Rb/Sr analyses of the Tadhak complex (see Table 1).

limestone and dolomite typical of the Upper Proterozoic of the Taoudenni Basin and of some locally derived metaquartzite pebbles (Timetrine). The absence of material from the east (central Iforas) suggests that these sediments were deposited after erosion of the Pan-African mountain belt and peneplanation, a $^{39}\text{Ar}/^{40}\text{Ar}$ cooling age of 520 Myr having been obtained on a blue amphibole from the Pan-African foreland nappes bordering the graben to the west (H. Maluski, unpublished data). It seems reasonable, therefore, when taking into account the close spatial relationships between the dated alkaline intrusions and the graben, to ascribe the pull-apart graben horst tectonics to the Permian. This time relationship has now been partly confirmed by the discovery of fenitized alkaline rocks in the Tesoffi conglomerates (J. F. Sauvage, personal communication). The persistent mobility along the suture zone has been noted 1,000 km further north with the Saoura trough filled with over 6 km of Palaeozoic sediments located close to the Pan-African suture and affected by open folds of Upper Permian age¹¹. More recent mobility of the suture zone is shown by the presence of over 1,500 m of Cretaceous sediments in the region of Gao.

We conclude that there is a reasonable probability that a Lower Permian age can be assigned to the undersaturated complexes (including carbonatites) of the Tadhak province. This may relate to a widescale episode of rifting in Pangaea whose manifestations elsewhere include alkali magmatism in, for example, Corsica¹², the North Sea and southern Norway. The location of the province along the edge of the West African craton in relation to a pre-Cretaceous graben indicates the presence of hidden Upper Palaeozoic rifts beneath the extensive Mesozoic cover and may explain certain features of the subsurface geology of the Taoudenni basin.

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Hydrodynamic instabilities and photochemical reactions

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Although many photochemical structures obtained in irradiated solutions have been investigated recently^{1–4}, the origin of these patterns has remained unclear. Evidence has been presented which suggests that these structures are due to evaporative cooling. Using the Schlieren technique, photographs of prepatterns have been obtained before illumination and we show here that these prepatterns are associated with convective motions in the bulk. As a result, the hydrodynamic motions must be given proper consideration in the interpretation of spatio-temporal chemical patterns. The photochemical reaction also provides a useful way of visualizing slow convective motions.

In contrast to oscillatory chemical reactions, there are few experimental examples of stationary spatial chemical structures^{5–10}. The origin of these structures remains unclear because their characterization is difficult. Indeed such concentration waves must be produced in unstirred reactors, thus preventing any feeding. Because of the consumption of the reagents the system therefore tends to drift in parameter space, and the structures are then at best quasi-stationary. These reactions are, furthermore, complex and their mechanisms are usually not well understood. However, a series of photochemical patterns occurring during photolysis of halogen compounds has been reported by Avnir^{2,3} and similar patterns have been found⁴ in photochemical reactions of simpler mechanisms. These appear when a 1–7-mm layer of an aqueous solution of chromogen (irreversible) or an organic solution of photochromic compound (reversible) is irradiated in a 70-mm Petri dish. First, a homogeneous, very thin layer of coloured product appears in the vicinity of the illuminated surface and thereafter breaks down into inhomogeneous zones, whether the irradiation is made from above or below. These patterns appear not to be very sensitive to the chemical nature of the colour developers but are strongly dependent on the solvent in which they emerge. Evaporation of the latter has been shown to play a crucial part in the onset of such structures, and it is well known that evaporative cooling¹¹ can induce convective motions in fluid layers. We now report Schlieren technique¹² observations of clean striations both on the free surface and in the bulk. Such striations are already present before irradiation (prepatterns) (Fig. 1) either in water or in organic solutions. Prepatterns have also been obtained in the pure solvents used in these photochemical reactions (CCl_4 , CHCl_3 , CH_3OH , C_6H_{12} and so on). If the evaporation is suppressed by covering the Petri dish, the structures disappear. Moreover, the patterns revealed under irradiation coincide with surface and bulk prepatterns.

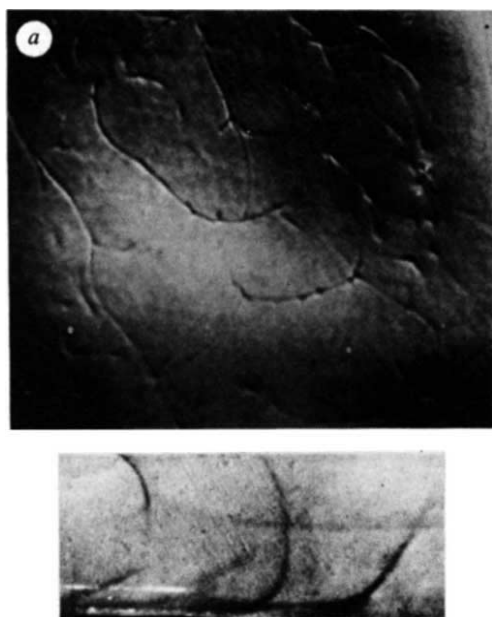


Fig. 1 *a*, Surface pattern in Möckel's reaction before irradiation. *b*, Bulk patterns in an aqueous solution of chromogenic developer before irradiation.

The following experimental facts strongly suggest that these patterns and prepatterns are associated with convective motions in the layer. (1) There exists a linear relationship between the average wavelength of the structures and the depth of the layer (Fig. 2). (2) When one deposits a layer of ink (green) on the bottom of a Petri dish filled with an aqueous solution of chromogenic developer under irradiation, one observes red sinking vermiculated rolls with green ones rising in between (Fig. 3).

The leading mechanism, therefore, seems to be the evaporative cooling and the coloured product formed in the irradiated layer is advected on the convective rolls.

Gravimetric measurements of the evaporation rate allow an estimation of the temperature gradient across the fluid layer. For water and methylbenzene we find respectively 1°cm^{-1} and $1.25^{\circ}\text{cm}^{-1}$. Using these values we have calculated the critical depth for surface tension (h_c^{st})- and buoyancy (h_c^{b})-driven instabilities:

	Water	Methylbenzene
h_c^{st} (mm)	0.8	0.7
h_c^{b} (mm)	4.7	2.7

Structures appear only for $h > h_c^{\text{st}}$.

As a consequence the conditions for the onset of convective motions are satisfied in all our experiments. For thicknesses $< h_c^{\text{b}}$ the surface tension forces are more effective than buoyancy effects, whereas both mechanisms reinforce one another for larger depth.

Any surface reaction (such as photochemistry, reaction at gas-liquid interfaces¹³, vaporization and adsorption) yielding or consuming a coloured product could provide a way for visualizing convective motions.

A striking example is provided by the system $\text{Ru}(\text{bipyridine})_3^{2+}/\text{methylviologen}^{2+}/\text{triethanolamine}$ ($\text{pH}=8$). With visible light the deep blue cation radical of methylviologen is formed thus revealing the prepatterns. After completion of the photochemical reaction the light source is turned off and the solution is homogenized by stirring. Because the cation radical is sensitive to oxygen, yellow reverse patterns on a deep blue background are thereafter observed resulting from the slow surface reaction of the cation radical with oxygen.

More generally a chemical reaction exhibiting a complex nonlinear kinetic network: for example, bromate⁶⁻⁸ or glycolytic⁹ oscillators, can give rise (in addition to spatio-temporal

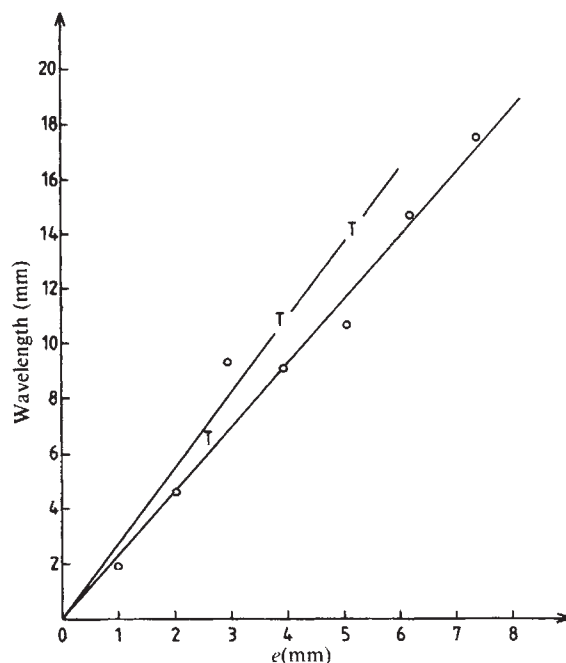


Fig. 2 Linear relationship between the pattern wavelength and the thickness of the fluid layer (e). O, Chromogenic developer in water; T, mercury dithizonate in methylbenzene.



Fig. 3 Surface patterns showing convective movements in an aqueous solution of chromogenic developer during irradiation. ↓, Down; ↑, up; R, red; G, green.

centre waves, the origin of which is purely chemical) to quasi-stationary mosaic striped structures. The shape of such mosaic structures has a striking similarity to that of the prepatterns. Here again their origin lies in the coupling with hydrodynamic motions. Moreover, the striations can induce the localization of centre waves. Similarly, in the case of photochemical reactions displaying oscillatory behaviours^{14,15}, it has also been shown that convective motion (eventually time dependent) has an important role. However, in the latter example the height of the system exceeds its radius and the corresponding convective behaviour is rather different.

We conclude that not all the structures we have studied, including those arising during Möckel's reaction¹, are induced by light. The photochemical reaction merely serves to reveal the prepatterns induced by evaporative cooling. Having characterized similar prepatterns in the solutions used in most of the photochemical reactions described by Avnir, we think that the same phenomenon is responsible for the onset of structures in those systems. Note that the simplicity and the flexibility of photochemical imaging¹⁶ could provide an alternative technique for studying the evolution, in real time, of convective patterns particularly in thin layers where standard optical methods are less efficient.

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Anthropogenic tritium in South Atlantic bottom water

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By observation of the invasion of man-made substances into the oceans, we learn about the nature and rates of mixing and flow. One of the more useful of these tracers is tritium (^3H), which was produced by the atmospheric thermonuclear weapons tests of the 1950s and 1960s and which entered the oceans in a pulse-like fashion, totally dominating the natural background. The production and entry of this radioisotope ($t_{1/2} \sim 12.5 \text{ yr}$)¹ into the ocean took place largely in the Northern Hemisphere, so that corresponding concentrations of tritium in the Southern Hemisphere are 5–10-fold smaller. Unfortunately, this means that the dynamic range (the ratio of maximum observable to minimum detectable concentrations) of tritium is limited for southern hemispheric work. If the minimum detectable quantity (that is, the detection limit) of tritium could be reduced, the evolution of this transient tracer would still provide valuable insight into mixing and advection. By increasing sample size and altering sample handling techniques, we have now extended the detection limit by more than an order of magnitude, and have detected what we believe to be man-made tritium in Antarctic bottom water at about 40° S in the western South Atlantic.

The ^3He regrowth technique for measuring tritium² involves the removal of dissolved gases and storage of the water sample *in vacuo* (usually for 1 yr). Extraction and measurement of the grown-in daughter, ^3He , provides a measure of the parent tritium content. The minimum detectable quantity of tritium is limited by the minimum detectable quantity of grown-in ^3He ($\sim 1.2 \times 10^{-16} \text{ cm}^3$ (STP) in our laboratory), so that the minimum detectable tritium concentration is inversely proportional to the storage time and the mass of water degassed. A typical 45-g sample stored for 1 yr has a tritium detection limit of $\sim 0.03 \text{ TU}$ (1 TU = 1 ^3H atom per 10^{18} H atoms). Increasing sample size and/or mass is simply a question of logistics. We obtained samples from 10-l Niskin bottles, transferring them to 4-l sample bottles which had been previously baked at 150 °C for 6–8 h in an argon atmosphere, filled with argon and sealed. One litre of each sample was transferred under argon atmosphere to 2-l aluminosilicate (low He permeability glass) vessels, vacuum degassed and stored for 4 months. The ^3He grown-in during the period was head-space extracted and analysed on a He-isotope mass spectrometer³ to an accuracy of $\pm 0.5\%$, $\pm 1.2 \times 10^{-16} \text{ cm}^3$ (STP) ^3He . Standardization was against air, using an He abundance of 5.24 p.p.m.v. and $^3\text{He}/^4\text{He} = 1.384 \times 10^{-6}$. Despite the short incubation times, the detection limit was 0.003 TU (hereafter reported in mTU). Extension of the storage time to a full year would reduce the detection limit to 1 mTU.

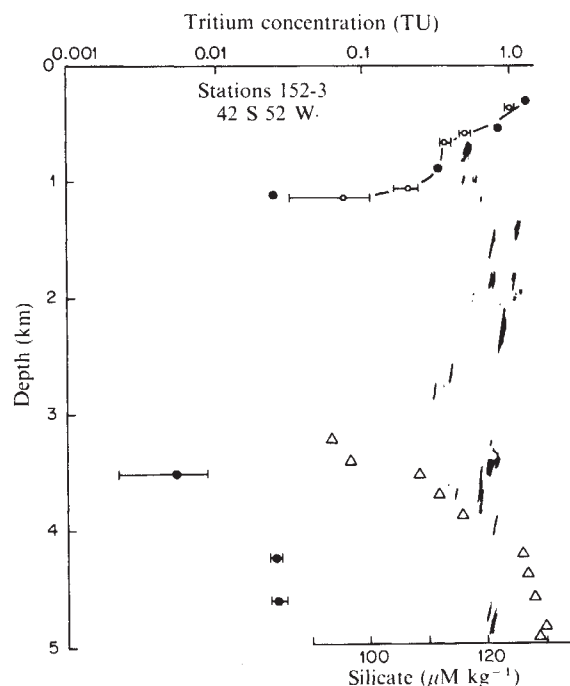


Fig. 1 Tritium concentration versus depth for a location in the South Atlantic near 42°S, 52°W. The tritium is reported in TU and is on a logarithmic scale. The closed symbols are large-volume (1 l) tritium samples measured by ^3He regrowth. The open symbols correspond to 'conventional' 45-g samples also measured by ^3He regrowth. Where error bars are not shown, uncertainty is less than symbol size. A partial silica profile (in $\mu\text{M kg}^{-1}$) is included for comparison.

We report here tritium analyses on samples taken from a station at approximately 42° S, 53° W in the South Atlantic. The station is situated at the poleward edge of the subtropical (wind driven) gyre, so that the shallow (<1 km depth) waters are probably representative of the gyre as a whole. We chose the station near the western boundary to capture a strong Antarctic Intermediate water influence, but placed it such that it was at the centre of what is believed to be a relatively intense bottom water recirculation within the Argentine Basin⁴. The data are shown as a profile versus depth in Fig. 1, with tritium reported on a logarithmic scale. The error bar is 1 s.d. of measurement, and where not shown is smaller than the size of the symbol. Some of the samples (characterized by the larger uncertainties at the greater concentrations) were the smaller, 45-g samples. For comparison, a partial silicate profile is also shown in the lower part of Fig. 1.

In the top 700 m, there is an exponential tritium decrease with depth associated with increasing isolation from the surface through the main thermocline. This trend, although scaled down by comparison, is consistent with observations of tritium profiles in the Northern Hemisphere. The decrease is interrupted at $\sim 900 \text{ m}$ by an inflection (local maximum) which corresponds to the depth of the Antarctic Intermediate water salinity minimum. We interpret this as the equatorward spread of tritium-tagged subpolar water from the polar front regions. Again, we see a similar feature in the North Atlantic⁵. Below this, the decrease resumes into the North Atlantic deep water (NADW), which we conjecture to be essentially tritium free. Unfortunately, several samples were lost due to breakage on the ship so that we do not have data for the mid-depths. However, we see the tritium concentrations increase near the bottom, from a value of $5 \pm 3 \text{ mTU}$ in what is predominantly NADW to $24 \pm 3 \text{ mTU}$ in Antarctic bottom water (AABW). The lowest concentration observed (5 mTU) sets an upper limit on potential contributions due to any contamination suffered by the samples, but consideration of hydrographic and nutrient data clearly shows that the signal could easily be explained as

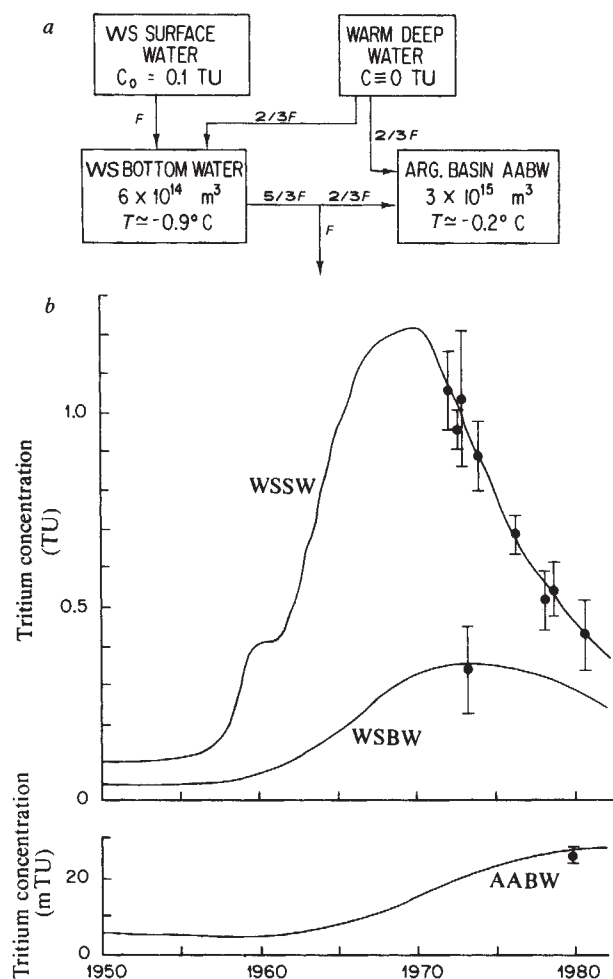


Fig. 2 A simple hydrologic model of Antarctic bottom water formation (a) and the model results for the time evaluation of tritium in the three important water masses (b). The data points are literature values before 1975 and unpublished results from this laboratory after 1975.

an upward mixing of tritium from the AABW. Some sampling at intermediate depths was performed, where smaller tritium concentrations are expected. Unfortunately, these samples were lost due to breakage in the ship's hold. We can, however, estimate blank contributions due to sample storage and handling.

Shipboard transfer was achieved under an 'argon blanket' so that the amount of water vapour exchanged is probably $\ll 100$ mg. At ambient shipboard tritium concentrations of < 10 TU, this leads to a maximum contamination of 0.25 mTU. Contamination due to transpiration through the bottle cap during storage can be estimated⁶. For a storage time of 1 yr and ambient tritium concentrations of 15 TU, we obtain an additional contribution of 0.25 mTU. Finally, the samples are transferred under an argon atmosphere into a vacuum-degassed and dried container. An upper limit to the blank contribution here is 0.1 mTU. The total estimated blank, therefore, is < 0.6 mTU.

Although we are dealing with a limited data set, the station is located so that it should be representative of a larger area. The shape of the tritium profile near the bottom is convincingly similar to the silica profile, so we are tempted to assume that the concentrations observed are characteristic of AABW in the Argentine Basin. We must then ask whether the concentrations observed are consistent with reasonable ventilation rates for AABW.

To do this we construct a simple hydrographic model of AABW formation which is, to within an unknown constant, consistent with hydrographic and stable isotope data. The

scheme constructed is shown in Fig. 2a. We start with Weddell Sea surface water (WSSW) which mixes with warm deep water (mixing ratio 3:2 as estimated from stable isotopes⁷) to form Weddell Sea bottom water (WSBW). Part of this water enters the Argentine Basin after mixing in transit again with warm deep water. Being very old, the warm deep water is essentially tritium free, so that the only tritium input to the system is via the WSSW.

To model the time history of the tritium concentration of WSSW, we apply the tritium hydrologic formulation used by Weiss *et al.*⁸ and incorporate the first order mixing/removal rate for removal of tritium from the surface mixed layer.

$$\frac{dC_s}{dt} = \left[P + E \frac{1}{\alpha} \frac{h}{1-h} \right] C_p - E \frac{1}{\alpha(1-h)} C_s - \lambda C_s - \gamma C_s$$

where C_s and C_p are the surface water and precipitation tritium concentrations, respectively, h is the relative humidity and P and E are the precipitation and evaporation rates, λ is the decay constant and γ is the removal rate. Values for E and P that would be appropriate to the calculation are probably zonally averaged values for the latitude band 50 – 60°S , inasmuch as the circumpolar current serves to homogenize surface waters on a time scale of a few years. We therefore used 0.35 and 0.85 m yr^{-1} respectively, and $h = 0.5$. Observation of the time decrease of tritium from 1973 (GEOSECS) to 1980 (ISOS) yields an estimate of $\gamma = 0.2 \text{ yr}^{-1}$ for circumpolar surface water. From this one can compute a preanthropogenic level of 0.1 TU for WSSW and time history curves as shown in Fig. 2b.

The surface water curve compares well with the data, in part because of the underdetermined nature of the calculation. The curve differs slightly in amplitude from the curve of Weiss *et al.*⁷ because their calculation was based on a local hydrological model for tritium. Considering the qualitative nature of this calculation, the differences are not significant. The choice of $F = 1 \times 10^6 \text{ m}^3 \text{ s}^{-1}$ yields a response curve for the WSBW which is consistent with the one data point, and the corresponding AABW curve is not inconsistent with the observations reported here.

The production rate of WSBW implied by this calculation ($\sim 2 \times 10^6 \text{ m}^3 \text{ s}^{-1}$) is consistent with, but at the lower end of, estimates made by others^{7,9}. The model is necessarily crude and underconstrained, due to the present lack of tritium data. Nonetheless, it does show that the tritium levels we observed in the Argentine Basin AABW are not inconsistent with accepted formation rates of Antarctic bottom water. It is interesting that within the framework of this simple model, the natural ^3H component in AABW is about 4 mTU, or $\sim 15\%$ of the observed signal. Conversely, we could argue that the tritium levels we observe here in the AABW are sevenfold higher than natural water levels would suggest. Also, the model predicts further increases in AABW, but shows decreases in WSBW and WSSW. Further measurements in these water masses could provide critical constraints and direction in reformulating the simple model used here.

Thus, we have observed significant levels of tritium in the bottom water of the Argentine Basin; these levels are consistent with a simple model of inclusion of bomb-produced tritium during AABW formation.

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Palladium and nickel in north-east Pacific waters

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Platinum group metals have not previously been analysed in seawater. However, they have unique chemical properties, such as their various oxidation states and their strong tendencies for complex formation¹, and have been used as a potential indicator of the presence of meteoritic material²⁻⁴. Moreover, they may have been dispersed about the environment from their use in catalytic converters for internal combustion engines, especially palladium⁵. Here I report the first palladium profiles in marine water and sediment columns. Palladium concentration in the water column of the north-east Pacific increases from 0.18 pmol kg⁻¹ at the surface to 0.66 pmol kg⁻¹ in deep waters. There is a strong co-variance between palladium and nickel, which implies similar biogeochemical pathways. Significant palladium enrichment in recent sediments from the Palace Moat, Tokyo, Japan, is observed and is perhaps a consequence of palladium usage in automobiles.

Water samples were collected in November 1982 at Station I (15°40' N, 107°20' W; water depth, 3,350 m) using Go-Flo bottles hung on Kevlar wire, and at Station II (32°37' N, 118°08' W; water depth, 2,050 m) using Niskin bottles with Teflon-coated internal springs hung on steel wire. The samples were acidified by adding 2 ml of 8 M HNO₃ per litre of seawater and stored for 1 week in conventional polyethylene bottles which had been cleaned with 3 M HCl at 60°C. Laboratory experiments with ¹⁰³Pd tracer indicated that there was no loss of palladium to the container walls in these conditions over a 3-month period. The samples for dissolved palladium assay were passed through 0.4-µm Nuclepore filters before acidification.

The analyses were made by graphite furnace atomic absorption spectrometry after a series of separation and concentration steps. The first step involved isolation of palladium as a cyanide complex from a large volume of seawater onto a small volume of anion exchange resin and subsequent elution with hot 14 M HNO₃. The eluent was evaporated to dryness, redissolved with 1.5 M HCl, and quantitatively transferred to a 1-ml Eppendorf pipette tip whose open end was sealed with parafilm. Finally, palladium was adsorbed on a single anion exchange resin bead by placing the bead in the solution and rotating the pipette tip for at least 12 h. Determination was carried out by inserting the bead itself into a graphite furnace AAS and burning (details of the method will be reported elsewhere). The detection limit using 2 l of water is about 0.11 pmol kg⁻¹; the precisions (1σ) are 11 and 7.2% at 0.6 and 1.2 pmol kg⁻¹ of palladium, respectively. Typical blanks ranged from less than the detection limit to 0.18 pmol kg⁻¹.

Figure 1 shows the analytical results of palladium in north-east Pacific waters, together with those of nickel and nutrients for comparison. The distribution of palladium in the water column displays the nutrient-type surface depletion and deep enrichment observed for many other trace elements such as cadmium, nickel and zinc⁶⁻⁸. The concentration increases from 0.18 pmol kg⁻¹ at the surface to 0.66 pmol kg⁻¹ in deep waters (Fig. 1). These values are about an order of magnitude lower than those for inland waters of the USSR⁹. For seawater,

Table 1 Palladium in sediments from Palace Moat, Tokyo, Japan

Depth in core (cm)	Depositional period*	Pd (nmol g ⁻¹ dry wt)†
7-8	1972-73	0.43
9-10	1971-72	0.12
11-12	1970-71	0.094
22-23	1964-65	0.030
31-32	1960-61	0.021
44-45	1953-54	0.021
55-56	1948-49	0.020

* Determination by unsupported ²¹⁰Pb (ref. 17).

† The analytical procedure for sediment was modified from that for seawater. After total dissolution of sediment with hydrofluoric acid, palladium was isolated as a thiocyanate complex from acidic medium to avoid iron hydroxide precipitation. Cyanide salt was not used in this case due to hydrogen cyanide gas evolution. Reduction of iron(III) to iron(II) by ascorbic acid was also required for better separation from iron. The remaining steps were identical.

Rosman *et al.*¹⁰ recently reported 38 pmol kg⁻¹ as an upper limit in an Australian coastal water. This is the only literature value available.

Although the sample collection and treatment methods were different for Stations I and II, similar palladium profiles were obtained. The nutrient profiles were also similar, indicating the similar oceanographic characteristics of the two stations. Station I samples were filtered after collection by Go-Flo bottles on a Kevlar line whereas Station II samples were unfiltered and collected by Niskin bottles on a steel line. Two unfiltered samples were also collected by Go-Flo bottles at Station I and values obtained were comparable with those for filtered samples. These results show that palladium in seawater is primarily in a dissolved form (that is, the palladium that passes through a 0.4-µm filter), as is nickel¹¹, and that palladium contamination during sampling is insignificant. Despite extremely low palladium concentrations in seawater (18,000 times lower than nickel), relatively little contamination was experienced during analysis in the laboratory. This may be due to the rarity of palladium in the environment and the strong resistance of palladium metal to oxidation. There is a strong correlation between palladium and nickel in these waters, with a Pd:Ni ratio of 1:18,000 (correlation coefficient 0.89 for 17 samples). Whitfield *et al.*^{12,13} recently reported a strong correlation between an element's oceanic residence time and seawater-crustal rock partition coefficient (defined as the ratio of an elemental concentration in seawater to that in crustal rock). As palladium and nickel occur in the Earth's crust with a similar ratio, 1:75,000 (nickel and palladium crustal abundances are 0.75 µmol g⁻¹ (ref. 14) and 0.01 nmol g⁻¹ (ref. 15), respectively), the oceanic residence time of palladium should be about the same as that of nickel, 10⁴-10⁵ y (refs 6, 7). The similar behaviour of palladium and nickel in ocean waters is not surprising considering their similar ionic potential, their strong association in minerals¹⁶ and the fact that they belong to the same Group in the Periodic Table.

Although copper profiles were not obtained in the present work, they may also bear some similarities to the palladium profiles. Previous studies^{6,12} have shown that the copper concentration increases with depth to the same extent as nickel. However, there is a difference in the overall profile shape between copper and nickel, copper profiles generally exhibiting concave curvature as a result of scavenging in deep waters and remineralization from surface sediments, while nickel profiles are convex due to regeneration in deep waters. From the present results (Fig. 1), it is difficult to tell whether palladium profiles are more similar to the profiles of nickel or those of copper, due to the rather large standard deviations of the palladium data and the absence of deep water data. A single 3,250-m deep water sample collected near Station I (17°30' N, 109°00' W) in November 1981 was analysed and found to

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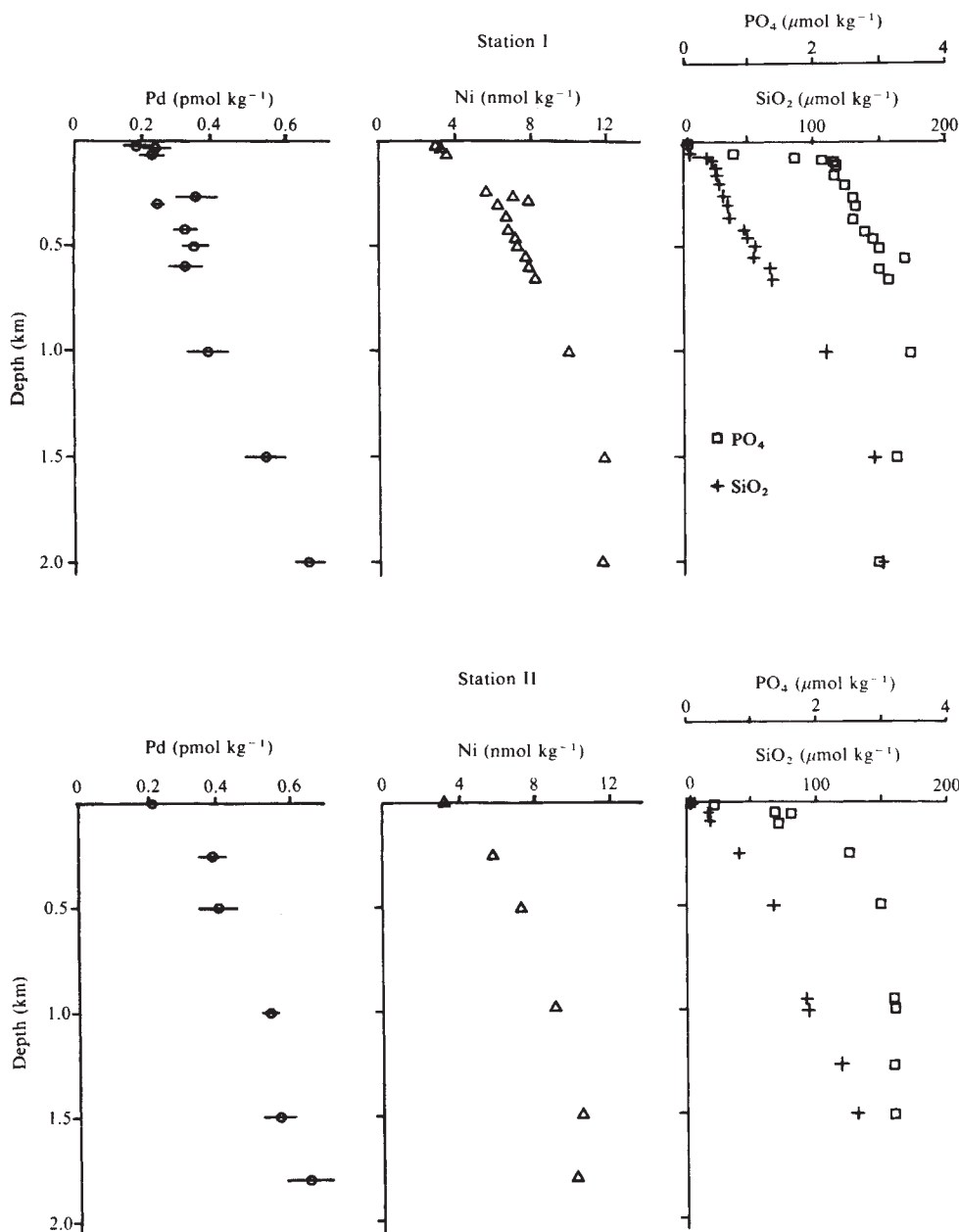


Fig. 1 Vertical profiles of palladium, nickel, silicate and phosphate in north-east Pacific Ocean. Palladium values are averages of triplicate analyses and analytical precisions (1σ) are indicated by error bars. Nickel analyses were done by graphite furnace atomic absorption spectrometry after APDC- CCl_4 solvent extraction¹¹. The precision for nickel was $\pm 5\%$.

contain $0.59 \text{ pmol kg}^{-1}$ of palladium. This value is about the same as those at mid-depth (1–2 km), indicating that palladium profiles are more similar to those of nickel. However, more profiles extending to the sea floor are needed to resolve the problem.

Table 1 gives the results of palladium analysis in sediments from Palace Moat, Japan¹⁷. Palladium contents in these sediments remained constant until the early 1960s and then increased rapidly. The sediment deposited between 1972 and 1973 contains 23 times more palladium than the deposits accumulated 30 yr earlier. It is interesting that at about the

same time⁵, palladium was introduced into automobile emission control systems as a catalyst. As the Moat is located in a heavily trafficked area, the excess palladium in recent sediment may originate from automobile exhausts. However, further study is needed to identify the sources of anthropogenic palladium.

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Comparison of ^{14}C and O_2 measurements of phytoplankton production in oligotrophic waters

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Present day estimates of primary phytoplankton production are derived almost exclusively from the results of a single method, the ^{14}C technique. ^{14}C methodology is amenable to routine measurement and also has the requisite sensitivity for the large expanses of ocean characterized by low autotrophic biomass. However, there is still great uncertainty as to its ecological interpretations; for example, whether it is measuring net or gross primary production. We report here the first comparison of planktonic photosynthesis based on ^{14}C assimilation with measurements of oxygen flux for an oligotrophic environment of ocean character. We conclude from the results that there is no evidence of persistent errors of any size, unique to the ^{14}C technique, in the measurement of gross production. We also infer, from a consideration of the rates themselves, that *in vitro* methodology is not biased with respect to *in situ* photosynthesis.

The oceans are thought to give rise to about one-third of global plant production¹⁻³. However, recent observations have suggested that the ^{14}C method is underestimating gross production by a factor of 2 to as much as 100 (refs 4-8). If rates of global production based on an extrapolation of ^{14}C data were found, for example, to be low by a factor of 10, one consequence would be a revision of our present notions of the distribution of productivity between land and sea; it would also mean that global productivity would be three to four times greater than presently estimated. If, on the other hand, the ^{14}C technique returns reliable data, it provides a maximum rate of net primary production. This would then call for a reexamination of the interpretation of the observations thought to be incompatible with existing rates derived from $^{14}\text{CO}_2$ incorporation measurements. As the ability of the ^{14}C technique to give accurate estimates of gross production seems to be worse in oligotrophic oceans, the technique may be most stringently tested in those locales⁹. Ideally, the ^{14}C technique should be verified against *in situ* changes in ΣCO_2 ; however, the precision of the ΣCO_2 determination is presently insufficient to allow this comparison to be made in the open ocean with any reliability. Recent refinements in dissolved oxygen determination, on the other hand, have increased the precision to a point where, with care, acceptable measurements of oxygen flux in the productive part of the water column can be made in oligotrophic waters¹⁰.

Measurements were made in the oligotrophic waters (chlorophyll *a* < 0.1 $\mu\text{g l}^{-1}$; nitrate concentration $\leq 0.03 \mu\text{M}$) off the west coast of the island of Oahu, Hawaii. Samples were collected from the upper mixed layer. Care was taken to avoid metal contamination during sampling by following the recommendations of Fitzwater *et al.*¹¹. All reported incubations were made in acid-cleaned, ~125 cm³, borosilicate bottles; neither the observed rates of photosynthesis nor simultaneous incubations in acid-cleaned polycarbonate containers¹² gave any indication that photosynthesis was inhibited in glass bottles, as has been reported^{11,13}. Except where noted, the samples were collected and processed before dawn, and placed in a deck incubator at 40% of ambient light density. One set of samples was incubated from dawn to dusk, a second set from dawn to

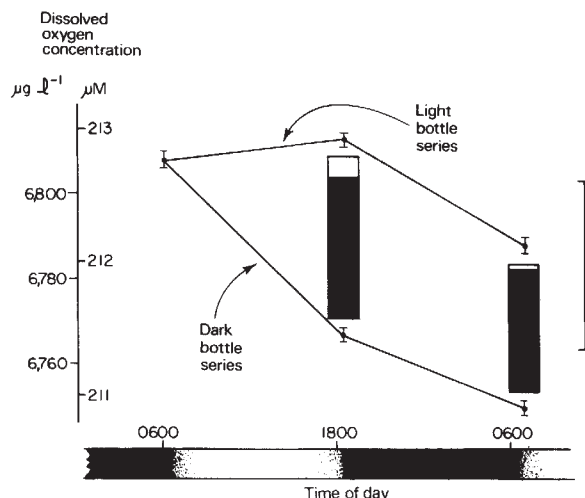


Fig. 1 Changes in dissolved oxygen concentration in dark and light bottles with time, compared with simultaneous measurements of $^{14}\text{CO}_2$ -determined carbon fixation in a sample taken on 21 September 1982. The histogram refers to the ^{14}C -determined rates: the solid portion is the particulate production, the open portion is the net extracellular products. The histograms are positioned to facilitate comparison with the dissolved oxygen changes. The lines connect the dissolved oxygen determinations; s.e.m. values are indicated. The oxygen and carbon scales have been adjusted for a *PQ* of 1.25, the vertical bar on the extreme right is equal to a carbon change of $1 \mu\text{mol l}^{-1}$.

the following dawn. The experimental protocol is evident from Fig. 1. At the end of the incubation, samples for the ^{14}C assay were collected on Millipore HA (0.45 μm) filters for liquid scintillation counting. The ^{14}C measurements were corrected for the net release of extracellular labelled photosynthetic products, which ranged over 3–10% of total carbon fixation. The dissolved oxygen determinations were made by a microprocessor-controlled photometric Winkler titration¹⁰. Chlorophyll *a* concentrations were determined by fluorometry on acetone extracts of particulate matter collected on Whatman GF/F filters, in initial samples and those incubated. There was no indication of marked decreases in chlorophyll *a* concentration during the incubation period (see refs 14, 15).

The data from the above studies and an earlier one are summarized in Table 1. Data from one station (Fig. 1) illustrate very clearly that the ^{14}C -determined rates are more closely related to gross than net production (terminology according to Strickland¹⁶). Respiration is comparable with photosynthesis over a 24-h period; thus, the system is more or less in material balance. The ^{14}C and oxygen methods are best compared by examining the molar flux ratio of gross oxygen production over the light period and $^{14}\text{CO}_2$ -determined CO_2 fixation over the same period. This ratio should approximate to the photosynthetic quotient (*PQ*). Although a wide range of *PQ* values have been reported¹⁷, stoichiometric considerations^{18,19} lead one to expect a range of 1.0–1.8. The observed molar ratios in Table 1 show no significant departure from this range. Ammonia is considered to be the predominant nitrogen source for the phytoplankton in areas of the type studied^{20,21}. If this is the case, the *PQ* should fall in a narrower range (1.0–1.3). Because the observed molar ratios in the present study do not lie significantly outside this range, we conclude that there is no evidence in the data that the $^{14}\text{CO}_2$ method seriously underestimates gross production, as measured by *in vitro* dissolved oxygen concentration changes.

This, however, does not necessarily resolve the question of the overall accuracy of either method, for both involve *in vitro* incubation, which has long been thought to introduce serious errors, resulting in underestimates of photosynthesis. The magnitude of the rates themselves we interpret as evidence for the

Table 1 Summary of oxygen and $^{14}\text{CO}_2$ -determined photosynthetic rates at a station off the west coast of Hawaii

Date	Chlorophyll <i>a</i> concentration ($\mu\text{g l}^{-1}$)	Daytime incubations			$^{14}\text{CO}_2$ -determined photosynthetic CO_2 fixation ($\mu\text{mol C l}^{-1}$)	Normalized photosynthetic rates (μg substrate per μg chlorophyll <i>a</i> per h)		Molar flux ratio (+ ΔO_2 /− ΔCO_2)
		Respiratory oxygen consumption ($\mu\text{mol O}_2 \text{ l}^{-1}$)	Gross photosynthetic oxygen production ($\mu\text{mol O}_2 \text{ l}^{-1}$)	Net 24-h oxygen production ($\mu\text{mol O}_2 \text{ l}^{-1}$)		Oxygen	Carbon	
5 August 1980	0.096	1.75±0.07*	1.06±0.28*	−0.69±0.26	0.70 ND	29.5	7.3	1.52
9 September 1982	0.092	1.08±0.11	1.12±0.11	+0.27±0.13	0.67±0.03	55.7	12.5	1.66±0.45
16 September 1982	0.084	1.06±0.07	1.88±0.05	−0.64±0.08	1.59±0.07	59.7	18.9	1.18±0.06
21 September 1982	0.085	1.29±0.04	1.44±0.04	−0.61±0.07	0.95±0.06	45.2	11.2	1.51±0.10
22 September 1982	0.077	0.37±0.13	0.92±0.11	−0.07±0.21	1.01±0.01	31.8	13.1	0.92±0.12

Station sampling positions were: 9 September 1982: 21°16' N, 157°55' W; 16 September 1982: 21°22.7' N, 158°13.4' W; 21 September 1982: 21°17.6' N, 158°08.9' W; 22 September 1982: as 21 September 1982; 5 August 1980: 39°30' N, 66°54' W. The sample taken on 9 September 1982 was incubated from 1130 h to dusk and that on the 5 August 1980 from dawn to dusk, the remainder from dawn to dusk. The precision limits given are the s.e.m.; characteristically 8–10 replicates were used for each oxygen determination and 4 for the ^{14}C . Except for the data for 5 August 1980, the respiratory oxygen consumption and gross oxygen production are calculated from the samples analysed at dusk; the net 24-h oxygen production is an independent measurement calculated from samples processed the following dawn. ND, not determined.

* 24-h incubation.

accuracy of the present *in vitro* measurements. The photosynthetic rates are high: the chlorophyll-normalized rates of carbon fixation are comparable with the maximum ones observed for cultures²², close to a theoretical assimilation number of 24 calculated by Falkowski²³. Thus, if our *in vitro* methods extensively inhibited photosynthesis, we would have to recognize assimilation numbers higher than any yet reported. The high chlorophyll-normalized rates may be viewed as circumstantial evidence that the photosynthetic activity of natural oligotrophic populations is not depressed by stress, induced by handling or containment.

We conclude that, although the data set is clearly limited, it provides no evidence for persistent errors, unique to the ^{14}C technique, when used for the determination of primary production of oligotrophic waters. We also conclude that the technique measures gross rather than net production. Similar conclusions have been drawn from studies in coastal and estuarine waters^{19,24}. In the present work we have encountered populations exhibiting high chlorophyll-normalized rates and it remains to be seen if the agreement between the two methods persists in oligotrophic water at low normalized rates. With the temperate water populations²⁴ we observed agreement between the $^{14}\text{CO}_2$ and oxygen methods at both high and low chlorophyll-normalized photosynthetic rates and thus we see no *a priori* reason to expect otherwise with oligotrophic populations.

The present data then appear to go some way to resolving a controversy that has existed since the introduction of the use of $^{14}\text{CO}_2$ for the measurement of primary production in the open sea. We note, however, that the rates we observed are higher than most reported values of production derived from the ^{14}C technique for waters of low chlorophyll *a* content^{25–27}, but are distinctly lower than the rates observed by Riley²⁸ (based on oxygen fluxes) for the northern Sargasso Sea. Two other studies in the vicinity of Oahu, Hawaii, recorded CO_2 -fixation rates comparable with our observations (ref. 29 and A. Cattell and D. C. Gordon, unpublished). Finally, we find no indication in the data that *in vitro* procedures seriously underestimate *in situ* rates of photosynthesis.

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Injection of GTP-binding protein or cyclic GMP phosphodiesterase hyperpolarizes retinal rods

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Light-induced activation of cyclic GMP phosphodiesterase (PDE) in the outer segments of vertebrate rod photoreceptors has been suggested as a step in the transduction process linking the absorption of light by rhodopsin with a hyperpolarization of the plasma membrane^{1–3}. Activation of PDE is mediated by a 'GTP-binding protein' (G). As a result of interaction with a rhodopsin photoproduct (possibly metarhodopsin II₃₈₀), this GTP-binding protein exchanges a previously bound GDP for a GTP^{4,5}. This 'GTP-charged' protein (G^{GTP}) is thought to activate PDE through an interaction in which an inhibitory protein is released from PDE^{6–8}. Assays of PDE activity *in vitro* have demonstrated that the hydrolysis of cyclic GMP

occurs within milliseconds of light onset⁹, which suggests that this process is rapid enough to be an intermediate step in transduction. To test this idea electrophysiologically, we have injected purified GTP-binding protein that was binding a hydrolysis-resistant analogue of GTP, guanylyl imidodiphosphate (p(NH)ppG), termed $G^{p(NH)ppG}$, as well as partially purified PDE, into toad rod outer segments while recording membrane voltage. We report here that injection of either of these biochemically active proteins induces a reversible hyperpolarization of the rod membrane, while injection of inactive proteins seems to have no effect on membrane voltage.

Double-barrel micropipettes were used to impale outer segments of red rods in the dark-adapted, isolated retina of the toad, *Bufo marinus*^{10,11}. Using a modification of the method of Oakley and Pinto^{12,13}, one barrel was used as a conventional microelectrode to record membrane voltage and the other barrel was used to pressure-inject various protein-containing solutions into the rod outer segment (ROS). GTP-binding protein was purified from toad ROS membranes by extraction from bleached membranes using either p(NH)ppG or GTP (Fig. 1A, B)¹⁴. PDE was partially purified by extensive washing of the bleached membranes with GTP in moderate ionic strength buffer, followed by low ionic strength washes (Fig. 1C)^{15,16}. This method of extraction yielded PDE that was partially activated, due to release of inhibitor protein from PDE by the earlier GTP washes^{6,8,17}. Aliquots of the proteins to be injected were added to ROS membranes in darkness and their abilities to stimulate cyclic GMP hydrolysis were assayed¹⁸. Both $G^{p(NH)ppG}$ and PDE significantly increased cyclic GMP hydrolysis above basal levels, while G^{GDP} was inactive (Fig. 1).

The effects of injection of $G^{p(NH)ppG}$ into a rod outer segment are shown in Fig. 2. After impalement of the rod, the membrane voltage (V) was allowed to stabilize, and the rod was stimulated by flashes of light (control photoresponses, Fig. 2A (top), B). Three injections of $G^{p(NH)ppG}$ were made with increasing pressure (Fig. 2B, C, D), each rapidly and reversibly hyperpolarizing the cell. After these injections, the photoresponse waveform was unaltered (Fig. 2A, bottom). A fourth injection (Fig. 2E), made with a much greater pressure, produced a large hyperpolarization that lasted until the micropipette became dislodged from the rod. Extracellular injection had negligible (<1 mV)

effect on electrode voltage (Fig. 2F). Qualitatively similar effects of injected $G^{p(NH)ppG}$ were obtained in 32 rods.

Figure 3 shows the effects of injection of partially purified PDE. Injection of PDE rapidly hyperpolarized the cell, and the hyperpolarization decayed with a time course similar to that of the photoresponse elicited before or after the injection. Extracellular injection had negligible effect on electrode voltage. Qualitatively similar effects of injected PDE were observed in 14 rods. Hyperpolarizations as large as 13 mV were elicited by injection of $G^{p(NH)ppG}$ or PDE.

We injected several proteins that did not significantly stimulate cyclic GMP hydrolysis above basal levels when added to ROS membranes *in vitro* (<5% of light activation). We attempted injections of G^{GDP} into 22 rods, and none of these injections caused significant hyperpolarizations. Injections of boiled $G^{p(NH)ppG}$, boiled PDE, or bovine serum albumin, which did not significantly increase cyclic GMP hydrolysis when added to ROS membranes, similarly had negligible effects on membrane voltage (data not shown).

We have now shown that biochemically active proteins, which

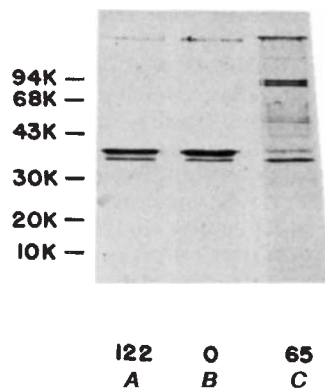


Fig. 1 SDS-polyacrylamide gel electrophoretic pattern and cyclic GMP hydrolysis activity of proteins injected. A, Purified GTP-binding protein extracted from bleached ROS membranes with p(NH)ppG, yielding $G^{p(NH)ppG}$. B, GTP-binding protein extracted with GTP (which is hydrolysed to GDP), yielding G^{GDP} . C, Partially purified PDE, which contains the protein of molecular weight 36,000 (36K) associated with the GTPase in addition to the 84K and 88K PDE doublet¹⁵. The numbers below each lane indicate the increase above basal levels of cyclic GMP hydrolysis, expressed as a percentage of light activation, observed on addition of the protein to ROS membranes in darkness ($G^{p(NH)ppG}$ and G^{GDP} , 1 mol per 100 mol rhodopsin; PDE, 1 mol per 40 mol rhodopsin); light activation was 21 mol cyclic GMP hydrolysed per mol rhodopsin-min (fully bleached membranes in the presence of 2 μ M p(NH)ppG). PDE also hydrolysed cyclic GMP in the absence of ROS membranes.

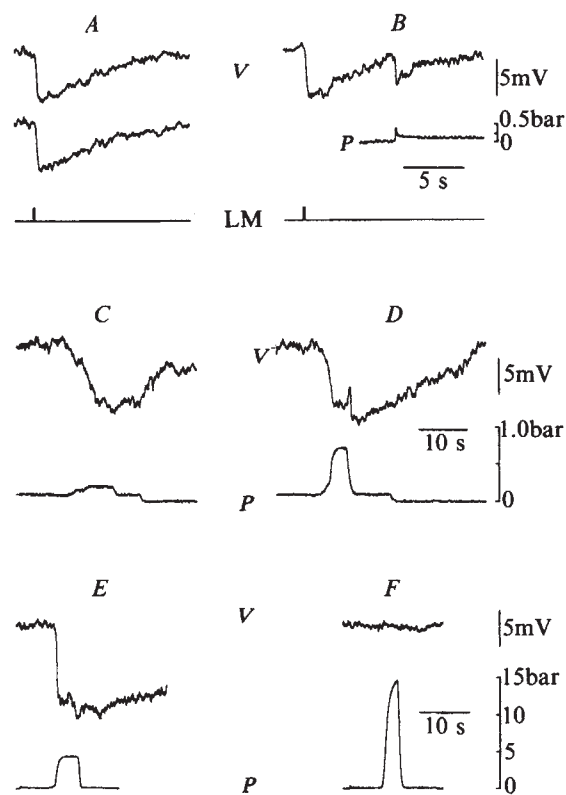


Fig. 2 Intracellular pressure-injection of $G^{p(NH)ppG}$ into a toad rod outer segment. Membrane voltage (V) was measured by the recording barrel (filled with 1.0 M potassium acetate, 25 mM MOPS, pH 7.1). Criteria for an acceptable penetration were (1) a resting value of V of at least -30 mV, and (2) a photoresponse with a 'plateau' of at least 10 mV. Criteria for an acceptable injection were that (1) the amplitude of a post-injection photoresponse was within 1 mV of a pre-injection photoresponse elicited by a flash of equal irradiance, and (2) after withdrawal of the micropipette from the rod, an extracellular injection produced negligible (<1 mV) change in electrode voltage. A, Photoresponses elicited before (top) and after (bottom) injections shown in B, C, D. Each stimulus was a 100-ms flash of 500 nm light, having an irradiance of 23 quanta $s^{-1} \mu m^{-2}$ (LM, light monitor). B-E, Responses to injections of a solution containing: 1 μ M $G^{p(NH)ppG}$, 22 mM MOPS, 4 mM dithiothreitol, 15% glycerol, pH 7.1. The solution filling the injection barrel was injected into the rod by applying pressure to the barrel^{12,13}. A pressure transducer was used to measure the applied pressure (P). During the course of records A-E, the resting value of V varied by <3 mV. F, Extracellular injection.

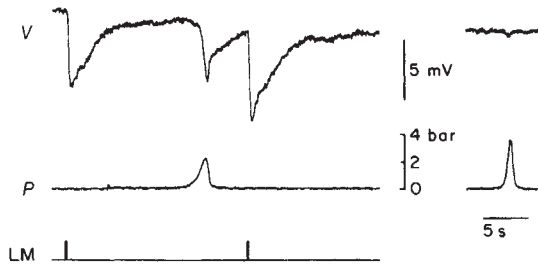


Fig. 3 Injection of PDE into a rod outer segment. The injection solution contained 3 μ M PDE (partially purified), 22 mM MOPS, 4 mM dithiothreitol, 15% glycerol, pH 7.1. The left-hand record shows injection into a ROS, while the right-hand record shows an extracellular injection.

mimic the effect of light on ROS cyclic GMP biochemistry *in vitro*, also produce rapid membrane hyperpolarizations when injected intracellularly. As $G^{p(NH)ppG}$ activates PDE irreversibly *in vitro*¹⁹, one might have expected a permanent hyperpolarization following injection of $G^{p(NH)ppG}$. However, inactivation of one or more steps subsequent to cyclic GMP hydrolysis could bring about a reversal of the observed hyperpolarization even in the presence of continued activation of the nucleotide cascade²⁰. A mechanism for independent inactivation of the light-sensitive conductance has been proposed²¹. An alternative explanation, which assumes that rod membrane potential is dependent on the level of cyclic GMP, argues that guanylate cyclase can synthesize cyclic GMP at an increased rate in order to compensate for the increased rate of hydrolysis²⁰. Guanylate cyclase has been estimated to be capable of synthesizing $>10^7$ cyclic GMP molecules per second in physiological conditions²².

It has been demonstrated recently that the dominant conductance in amphibian rod outer segments consists of light-sensitive Na^+ channels²³. This suggests that the protein-induced hyperpolarizations are probably due to modulation of the light-sensitive conductance. As light activates PDE via GTP-binding protein (as G^{GTP}), and injection of $G^{p(NH)ppG}$ or PDE produces membrane hyperpolarization, our data support the hypothesis that the activation of PDE by G^{GTP} and the resulting hydrolysis of cyclic GMP are intermediate steps in the pathway linking the absorption of light with the membrane hyperpolarization. We acknowledge support from NIH grants EY04364, EY04182 and EY07000, a grant from the Chicago Community Trust/Searle Scholars Program, and AFOSR C (F49620-83-C-0050).

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Conductance and dye permeability of a rectifying electrical synapse

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Electrical synapses provide a basis for efficient signal transmission in a wide variety of nervous systems¹. These synapses are composed of specialized cell-to-cell contacts known as nexuses² or gap junctions³ which mediate the direct transfer of ions and small molecules between adjacent cell interiors^{1,4} by way of intercellular channels embedded in the junctional membrane⁵. The crayfish giant motor synapse (GMS) was the first cell-to-cell junction clearly demonstrated to operate by an electrical mechanism⁶. Current applied to the presynaptic lateral giant (LG) axon or to the neurite of the postsynaptic giant flexor motoneurone (MoG) spreads passively through the synapse into the adjacent neurone^{6,7}. Each GMS behaves like an electrical rectifier: its conductance is high when LG is positive with respect to MoG, and decreases dramatically when the sign of the trans-synaptic voltage is reversed^{6,7}. We have now examined GMS conductance and dye permeability at thoracic and abdominal levels of the crayfish nerve cord. At both levels, values of GMS chord conductance fit a simple Boltzmann model in which the conductance of individual synaptic channels is assumed to be voltage dependent. Moreover, thoracic synapses display higher limiting conductances than do those at an abdominal level, apparently as a result of their larger size. We also find that synapses at both locations are permeable to the fluorescent dye Lucifer yellow, even in conditions where electrical conductance is low. These results provide a framework for understanding the operation and functional limits of rectifying electrical synapses, and illustrate that dye permeability can be associated even with their relatively low conductance condition.

The structure of the MoG was determined using cobalt chloride⁸ and Lucifer yellow⁹ staining. Giant motor synapses are found at regions where LG axons and MoG neurites overlap (Fig. 1) and occur on both sides of the nerve cord below most thoracic and abdominal ganglia¹⁰. Each GMS is further characterized by the presence of numerous short dendrites which protrude from the MoG neurite along its length of apposition with LG¹¹. These dendrites form gap junctions with the LG axon¹² and thereby represent the actual sites of cell-to-cell coupling. Giant motor synapses at the level of the eighth thoracic ganglion were five to six times larger than those at the second abdominal ganglion, as assessed by the length of cell-to-cell contact and the area of MoG dendrites (Fig. 1, Table 1).

The functional consequences of these striking differences in GMS size were examined using intracellular recording techniques (Fig. 2). Impulse transmission was unidirectional for synapses at both thoracic and abdominal locations. Action potentials generated in the LG were rapidly and efficiently transmitted to the MoG (Fig. 2a) whereas MoG action potentials produced only small, subthreshold depolarizations in the LG (Fig. 2a_{ii}). The transmission of steady-state potentials displayed a similar bias. At both locations, outward depolarizing currents applied to the LG spread freely across the GMS, producing substantial depolarizations in the MoG (Fig. 2b_i), while outward currents applied to the MoG produced more

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Fig. 1 Structure of giant flexor motoneurons; dorsal views, rostral at top. *a*, Drawing of a cobalt chloride backfill preparation showing a pair of MoGs at the level of the second abdominal ganglion. The borders of the nerve cord and giant axons have been sketched in. Note that each MoG soma (s) and axon (a) are joined by a neurite (n) which is devoid of dendrites within the ganglion. The neurites are widest near the motor nerve (r3) and, in this region alone, feature numerous short dendrites (d) which make synaptic contact with lateral (L) and medial (M) giant axons. These areas of apposition define the borders of each GMS. *b*, Lucifer yellow injection showing the structure of a MoG neurite located just below the eighth thoracic ganglion. The neurite was impaled with a microelectrode containing 4% (w/v) Lucifer yellow and the dye injected by iontophoresis^{7,9}. The preparation was then fixed in 10% formalin, dehydrated in ethanol, cleared in methylsalicylate and photographed as a whole mount using fluorescence microscopy⁹. Note the greater number of dendrites extending from the neurite and the longer length of LG-MoG contact at this thoracic GMS compared with the abdominal synapse depicted in *a*. Calibration bars, 200 μm .

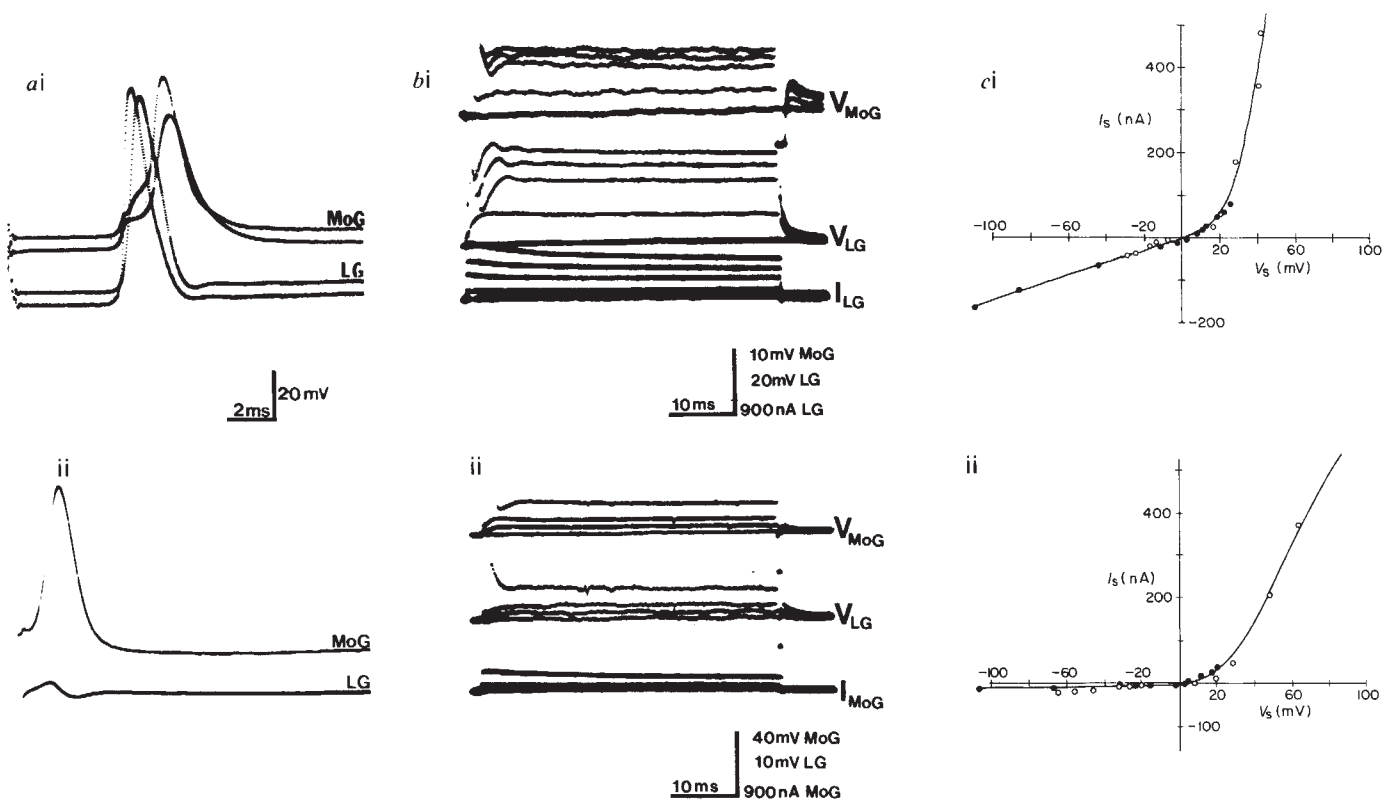
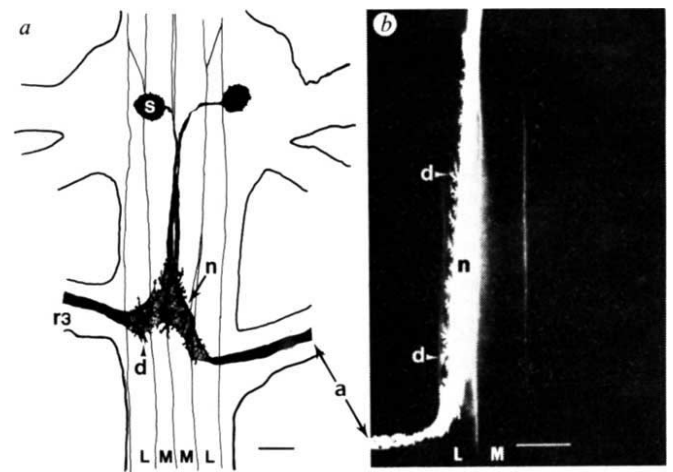


Fig. 2 Physiology of giant motor synapses. LG axon and MoG neurite were each impaled with two microelectrodes, one for voltage recording and one for current injection^{6,7}. *a*, Unidirectional impulse transmission, thoracic GMS: LG action potentials generated by nerve cord stimulation were reliably transmitted to MoG(i), whereas a MoG impulse generated by r3 stimulation produced only a 4 mV depolarization in LG(ii). *b*, Transmission of steady-state potentials, thoracic GMS. Depolarizations are considered positive. i, Outward currents applied to the LG (I_{LG}) depolarized it directly (V_{LG}) and the MoG by spreading across the GMS (V'_{MoG}). ii, Outward currents applied to the MoG (I_{MoG}) depolarized it (V_{MoG}) and the LG (V'_{LG}). Hyperpolarizing currents were also applied to each neurone (records not shown). Note that the coupling ratio (V'/V) expressing the efficiency of transmission, is larger in i where $V_{LG} - V_{MoG}$ is positive (that is, positive V_s). *c*, The current-voltage relationship of a thoracic GMS (i) is shown for comparison with that of an abdominal GMS (ii). In both cases, the current crossing the synapse (I_s) has been plotted as a function of the applied trans-synaptic voltage (V_s) (circles) for each value of V' . Since LG and MoG had different resting potentials, the values of V_s and I_s were adjusted so that the origin of each plot corresponded to zero trans-synaptic voltage and current. The synaptic current producing V' was determined by assuming it to be identical to directly applied current which produced the same change in membrane potential⁶. This information was obtained separately in each experiment from the current-voltage relationships of LG and MoG⁶ (records not shown). To ensure that none of the applied current crossed the GMS during these measurements, V_s was made close to zero by adjusting the currents applied to the LG and MoG so that the resulting changes in potential in each were equal to within 1 mV²². Note, in both GMS current-voltage plots, that I_s depends on V_s but not how it is produced; LG polarizations (filled circles) and MoG polarizations (open circles) that produced similar values of V_s caused nearly the same currents to cross the GMS. Some or all of the slight deviation between open and filled circles may be due to voltage dependent nonlinearities in non-junctional membrane conductance introduced when the LG or MoG is depolarized. Solid lines were drawn by computer for each GMS experiment using equation (1). For the thoracic GMS depicted in i, the values of g_{min} , g_{max} , A and V_0 used in equation (1) were 1.497 μS , 18.000 μS , 0.107 mV^{-1} and 39 mV, respectively. Values of the same parameters obtained for the abdominal GMS shown in ii were 0.133 μS , 6.075 μS , 0.093 mV^{-1} and 28 mV, respectively.

attenuated depolarizations in the LG (Fig. 2bii). To compare the resistance to current spread across large and small synapses, the current-voltage relationships of eight thoracic and four abdominal giant motor synapses were examined. GMS current-voltage curves were constructed by plotting synaptic current (I_s) as a function of trans-synaptic voltage ($V_s = V_{LG} - V_{MoG}$) (Fig. 2c). Synapses at both locations displayed rectification; GMS chord conductance ($g_s = I_s/V_s$) was high when the LG was positive with respect to the MoG (that is, positive V_s) and decreased dramatically when the trans-synaptic voltage approached zero or was made negative. Consistent with their larger size, however, thoracic synapses typically displayed higher chord conductances at both positive and negative trans-synaptic voltages than did abdominal synapses (compare Fig. 2ci and ii).

The current-voltage data in every GMS experiment fit a simple model where the synaptic membrane is assumed to contain a number of junctional channels^{5,12}, each able to change its unit conductance rapidly depending on the value of V_s (ref. 13). The extremely fast nature of the change between high and low synaptic conductance states (Fig. 2b) suggests that it is mediated by rapid, voltage-sensitive gating^{5,13} or by a voltage-dependent modification in the size of an energy barrier within each channel¹⁴. While qualitatively similar results could be obtained from either mechanism, we considered only a gating scheme where the channels exist in either an open or closed state. If the channels obey a Boltzmann distribution where the ratio of open to closed channels is an exponential function of V_s , being low at negative V_s and approaching infinity for large positive values, then each synapse will display rectification similar to that seen experimentally. Using such considerations, the synaptic chord conductance will be given by

$$g_s = \{(g_{\max} - g_{\min}) / 1 + \exp[-A(V_s - V_0)]\} + g_{\min} \quad (1)$$

Expressions, similar to equation (1), were used previously to describe the voltage-dependent behaviour of channels in artificial and embryonic junctional membranes^{13,15}. In each GMS experiment, the minimum conductance ($g_{\min}$, Table 1) was obtained from the slope of the current-voltage relationship at negative V_s . The maximum conductance ($g_{\max}$, Table 1) as well as the voltage constant expressing the value of V_s at half-maximal conductance (V_0), and the constant reflecting the voltage sensitivity of g_s (A) were obtained from the current-voltage data at positive V_s using the iterative procedure described by Spray *et al.*¹³. Equation (1) accurately predicted the characteristic, nonlinear current-voltage properties of both

thoracic and abdominal giant motor synapses over the wide range of trans-synaptic voltages studied. As demonstrated by the examples in Fig. 2c, the correspondence of measured current-voltage values (circles) with those obtained using equation (1) (solid lines) was consistently very good.

The parameters used in equation (1) proved useful for making quantitative comparisons of thoracic and abdominal giant motor synapses. The value of the constant A , expressing the voltage sensitivity of g_s , showed little variation between synapses; the overall mean ($\pm$ s.e.m., $n = 12$) was $0.126 \pm 0.042 \text{ mV}^{-1}$. Values obtained for the voltage constant V_0 were slightly lower for thoracic than abdominal synapses; the means were $19 \pm 4 \text{ mV}$ ($n = 8$) and $26 \pm 1 \text{ mV}$ ($n = 4$), respectively. The difference in these values, however, was not statistically significant ($P > 0.1$, by Student's two-tailed t -test); the overall mean was $22 \pm 3 \text{ mV}$ ($n = 12$). These findings suggest that individual synaptic channels at both locations share a similar voltage sensitivity.

The sensitivity constant A obtained here is about half that for amphibian blastomeres¹³, while V_0 is about 1.5 times larger. In accord with their larger size and presumed larger number of junctional channels, thoracic synapses had higher limiting conductances than did abdominal ones (refer to $g_{\min}$, $g_{\max}$ in Table 1). The approximately fourfold difference in $g_{\min}$ values was in reasonably good agreement with the difference in synapse size at the two locations and was statistically significant ($P < 0.05$). The difference in $g_{\max}$ values at the two locations was less than twofold, however, and was not significant ($P > 0.1$). The latter finding can be resolved by considering that neither LG nor MoG is isopotential when the GMS current-voltage measurements are made at thoracic synapses⁷. From measurements of the length constants of the LG axon and MoG neurite, and consideration of the electrode placements used in these experiments, we have calculated that this non-isopotentiality will cause both $g_{\max}$ and $g_{\min}$ to be underestimated at thoracic synapses. The effect is more dramatic at large, positive V_s , causing $g_{\max}$ to be underestimated by about 55% while $g_{\min}$ is underestimated by only 17%. If the values of $g_{\max}$ and $g_{\min}$ obtained at thoracic synapses are corrected appropriately for the error introduced by non-isopotentiality (Table 1, asterisks) a nearly fourfold, significant difference is revealed between the $g_{\max}$ values obtained at thoracic versus abdominal synapses ($P < 0.01$). Thus, both thoracic and abdominal synapses show about the same degree of rectification ($g_{\max}/g_{\min} \sim \text{constant}$), regardless of large differences in size and limiting conductances. These results further suggest that the properties of individual synaptic channels at the two locations are similar, and that the

Table 1 Comparisons of thoracic and abdominal giant motor synapses

GMS location	GMS contact length (mm)	GMS area ($\times 10^{-4} \text{ cm}^2$)	$g_{\min}$ (μS)	$g_{\max}$ (μS)	$(R_{LG+MoG})^{-1}$ (μS)
Eighth thoracic ganglion	1.64 ± 0.05	3.68 ± 0.08	0.84 ± 0.17 $1.01 \pm 0.21^*$	10.78 ± 1.67 $23.79 \pm 3.68^*$	0.07
	(6)	(2)	(8)	(8)	
Second abdominal ganglion	0.25 ± 0.05	0.60 ± 0.08	0.23 ± 0.10	6.79 ± 0.50	0.01
	(3)	(3)	(4)	(4)	

Giant motor synapses were compared at the indicated levels of the nerve cord; all measurements are expressed as the mean $\pm$ s.e.m., followed below by the number of observations. Synapse areas and contact lengths were obtained from preparations stained with cobalt chloride or Lucifer yellow (see Fig. 1). The contact length is defined as the distance over which the LG axon and MoG neurite are apposed. The area of each GMS was estimated from the area of MoG dendrites in the region of cell contact, measured with a planimeter. Minimum synaptic conductances ($g_{\min}$) were obtained for each experiment from the slope of the current-voltage relationship at negative V_s using linear regression analysis. Maximum synaptic conductances ($g_{\max}$) were determined from the g_s data at positive V_s for each synapse. Briefly stated, equation (1) was expressed in linear form $\{B = \ln[(g_s - g_{\min})/(g_{\max} - g_s)] = A(V_s - V_0)\}$ and the value of $g_{\max}$ selected which gave the highest correlation coefficient (r) when B was plotted as a function of V_s ($r = 0.89 \pm 0.06$, $n = 12$). The constants A and V_0 (see text) represent the slope and voltage intercept of such plots. Conductance values for thoracic synapses are also shown corrected for non-isopotentiality imposed by the long length of LG-MoG apposition at this synapse (*). The summed resistance of apposed LG-MoG non-junctional membrane, equivalent in area to that of abdominal or thoracic GMS, was also determined and its reciprocal $(R_{LG+MoG})^{-1}$ compared with that of $g_{\min}$ for each synapse. The specific membrane resistances for the LG axon and MoG neurite were calculated using equations derived from linear cable theory¹⁴ after determining the length constant, input resistance and radius of each^{6,7,22}.

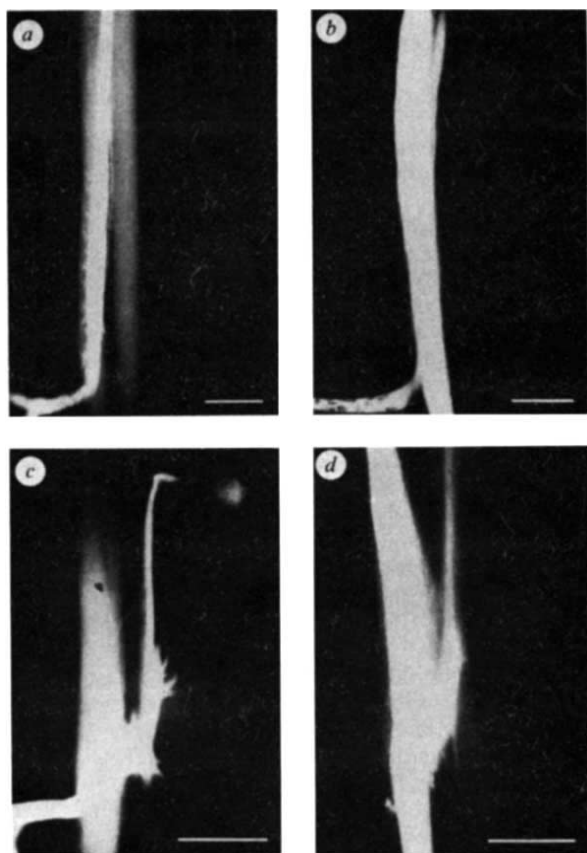


Fig. 3 Permeability of giant motor synapses. Lucifer yellow (4% w/v) was injected into the MoG (*a, c*) or LG (*b, d*) at thoracic (*a, b*) or abdominal (*c, d*) synapses using methods described in the text. Preparations are shown in the same orientation as in Fig. 1 and were photographed in the recording chamber 2–10 min after injection, without fixation. LG axons appear distorted in *b* and *d* because they were ligated above and below the GMS in order to restrict dye migration within the axon. Note that the dye has spread to the trans-synaptic neurone for each injection paradigm. Calibration bars, 150 μm .

higher conductances seen at thoracic synapses reflect a larger number of channels.

The permeability of giant motor synapses was assessed by examining Lucifer yellow transfer between the LG and MoG (Fig. 3). Lucifer yellow (molecular weight 457.2) was chosen as a marker because it is highly fluorescent and does not appear to penetrate non-junctional membranes⁹. To raise GMS conductance and thereby maximize the number of channels available for dye transfer, negatively charged Lucifer yellow molecules were iontophoresed while making V_s positive throughout the period of injection (15–40 min). This was achieved by injecting the dye into MoG with hyperpolarizing current pulses (100–500 nA) for one half of a 1-s duty cycle while depolarizing LG during the second half. In these conditions, Lucifer yellow spreads freely across the GMS. When nerve cords were examined 2–10 min after terminating injections in the MoG at either thoracic or abdominal synapses, dye fluorescence was clearly detected in the LG (Fig. 3*a,c*). Lucifer fluorescence was also sometimes seen in the medial giant axon (Fig. 3*a*), indicating that the dye can also spread to this electrically presynaptic neurone. To test if dye permeability is noticeably restricted when the GMS conductance is low, V_s was made negative throughout the injection by introducing Lucifer yellow into the LG while alternately depolarizing the MoG. These conditions did not block dye transfer, however, as Lucifer fluorescence was clearly visible in the MoG at both thoracic and abdominal synapses (Fig. 3*b,d*).

The experiments with Lucifer yellow demonstrate that the GMS, like other electrotonic junctions^{16–19}, is permeable to small dye molecules (Fig. 3). The failure to demonstrate fluorescein permeability at the GMS previously²⁰ may have been due to leakage of the dye across non-junctional membrane or to cytoplasmic dilution. We found that detection of cell-to-cell Lucifer yellow transfer was limited to living preparations viewed minutes after injection. Nerve cords allowed to soak in saline for 1–2 h showed less evidence of dye transfer, and those subject to fixation and dehydration showed none at all (Figs 3*a* and 1*b* depict the same preparation photographed before and after histological processing).

The observation that Lucifer yellow transfer occurs when the GMS is in either a high or low conductance state (Fig. 3) is somewhat surprising in light of results obtained from amphibian blastomeres where transjunctional voltages sufficient to cause a 10-fold drop in junctional conductance, also block dye permeability¹³. The detection of dye transfer from the LG to MoG in the low synaptic conductance state was probably facilitated by the MoG's smaller volume. Note from the GMS current-voltage curves (Fig. 2*c*), however, that substantial ionic coupling remains even when synaptic conductance is low. Specifically, the mean g_{min} values for both thoracic and abdominal synapses are an order of magnitude higher than the conductance of identical areas of apposed non-junctional membranes (Table 1). This finding suggests that some fraction of GMS channels are not voltage-sensitive, allowing the LG and MoG to be weakly dye coupled, as they are electrically coupled, even at negative trans-synaptic voltages. In spite of the observed qualitative independence of dye transfer on synaptic conductance, however, there is likely to be a significant quantitative difference in the permeability of low and high conductance synapses²¹. In particular, the rate at which Lucifer yellow diffuses between LG and MoG should depend on the relative number of open functional channels and therefore on GMS conductance. It will be useful to test this prediction by monitoring Lucifer transfer during the injections and comparing the diffusion rate for the dye when V_s is set at positive or negative values.

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Monoclonal antibodies to the acetylcholine receptor by a normally functioning auto-anti-idiotypic mechanism

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Recently¹ we described a procedure for preparing antibodies to the acetylcholine receptor (AChR) based on immunoglobulin idiotypes and on the hypothesis that, regardless of functional differences, macromolecules of the same specificity will show structural homologies in their binding sites. Antibodies were prepared in rabbits to a structurally constrained agonist of AChR, *trans*-3,3'-bis[α -(trimethylammonio)methyl]azobenzene bromide (BisQ)^{2,3}. These antibodies mimicked the binding specificity of AChR in its activated state⁴—agonists were bound with affinities that were in accord with their biological activities and antagonists were bound poorly. Rabbits were then immunized with a specifically purified preparation of anti-BisQ to elicit a population of antibodies specific for the binding sites of anti-BisQ. A portion of the anti-idiotypic antibodies^{5,6} produced in the second set of rabbits cross-reacted with determinants on AChR preparations from *Torpedo californica*, *Electrophorus electricus* and rat muscle. Moreover, several of the rabbits showed signs of experimental myasthenia gravis, in which circulating AChR antibodies are typically found. To devise a more direct route to monoclonal anti-receptor antibodies we based our strategy on acceptance of the concept of the anti-idiotypic^{5,6} network theory of Jerne⁷. According to this theory, injection of an antigen elicits, in addition to antibodies to the antigen, other populations that include anti-idiotypic antibodies directed at the combining sites of the antigen-specific antibodies. If the antigen-specific antibodies recognize a ligand of a receptor, then the anti-idiotypic antibodies should bind receptor. Thus, when a mouse is immunized with a bovine serum albumin conjugate of BisQ (BisQ-BSA)¹, it should be possible to expand populations of spleen cells that secrete antibodies which bind anti-BisQ and AChR, in addition to populations specific for BisQ. Fusion of the spleen cells with an appropriate myeloma line should yield monoclonal anti-AChR antibodies. Here we report the success of this approach and its implications.

BALB/cCr mice were immunized intraperitoneally (i.p.) with 0.1 ml of a 1 mg ml⁻¹ solution of BisQ-BSA conjugate¹ in complete Freund's adjuvant. Twenty-three days later, the mice were boosted i.p. with the same preparation. After 5 days, the spleen cells were collected and fused with a non-secreting myeloma line (P3 $\times$ 63-Ag 8.653)⁸ essentially by the protocol of Köhler and Milstein⁹ as modified by Sharon *et al.*¹⁰. Supernatants from hybridoma clones were obtained by a replica transfer technique¹¹ and screened for activity against BisQ-RSA, rabbit anti-BisQ and AChR (*Torpedo*) by an enzyme immunoassay¹, using peroxidase-labelled goat anti-mouse immunoglobulin as the second antibody.

As shown in Table 1 about 14% of the wells were positive for the antigen. Another set of wells, about 7.4% of the total, were positive for purified rabbit anti-BisQ; of these, about a third were also positive for AChR. Thus, not all anti-BisQ-reactive supernatants also bound receptor. Although a

significant number of the wells might have contained more than a single clone, and, certainly, the same clones could have been distributed in more than one well, we can conclude that immunization with BisQ-BSA induces a significant anti-anti-BisQ response.

Positive wells were then subcloned by the procedure of limiting dilution and several of the clones that produced antibody reactive with both anti-BisQ and *Torpedo* receptor were examined. Figure 1 shows titrations by enzyme immunoassay¹ of an (NH₄)₂SO₄ precipitate of a supernatant from one clone, F8-D5. The antibody produced by this clone was able to bind AChR of *Torpedo* as well as specifically purified rabbit anti-BisQ. This property was retained by clones obtained after two subsequent subclonings by limiting dilution. No attempt can be made to relate the respective binding affinities because of the characteristics of enzyme immunoassay procedures, in general. For example, there is no way to ensure that the same number of determinant sites of receptor and of anti-BisQ adhere to the plastic wells.

To confirm that the same monoclonal antibody bound receptor and anti-BisQ, reciprocal inhibition experiments were carried out. Figure 2 shows the results of an experiment in which the binding to anti-BisQ of an (NH₄)₂SO₄-precipitated supernatant of F8-D5 was inhibited by *Torpedo* receptor. At the highest concentration of receptor used, 67% inhibition was observed; 50% inhibition occurred with about 8 μ g of inhibitor in the conditions described. In experiments in which the amount of antibody was decreased fivefold to 2 μ g per well, 50% inhibition occurred in the presence of 0.9 μ g of *Torpedo* receptor. In a similarly designed series of experiments, 50% inhibition of the binding of F8-D5 to *Torpedo* receptor was accomplished with 3 μ g of purified rabbit anti-BisQ.

The binding of F8-D5 to *Torpedo* receptor could be inhibited by BisQ, 50% inhibition occurring at a concentration of 4×10^{-5} M. A similar concentration of BisQ caused 50% inhibition

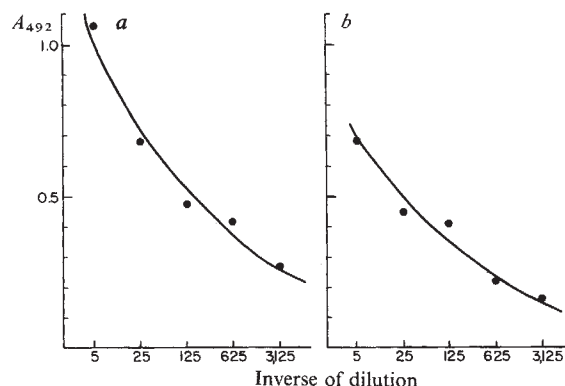


Fig. 1 Binding of *Torpedo* AChR (a) and rabbit anti-BisQ (b) by F8-D5. Wells of polystyrene plates (Corning) were coated with 150 μ l of either 400 ng ml⁻¹ purified rabbit anti-BisQ¹ in 0.1 M NaHCO₃, pH 9.3 or 3 μ g ml⁻¹ purified *Torpedo* receptor in the same buffer. Coating was accomplished by incubating for 2 h at 37 °C. After two washings with 0.01 M phosphate buffer–0.14 M NaCl, pH 7.2, containing 0.05% Tween-20 (PBS-Tween), the wells were exposed to the various concentrations of F8-D5 in PBS-Tween. After incubation at 37 °C for 2 h, the wells were washed three times with PBS-Tween and then filled with 200 μ l of 1:1,000 dilution in PBS-Tween of goat anti-mouse immunoglobulin labelled with horseradish peroxidase (Sigma). After incubation at 37 °C for 1 h, the wells were washed three times with PBS-Tween. Peroxidase was assayed by incubation with *o*-phenylenediamine dihydrochloride (7 mg in 10 ml 0.1 M citrate-phosphate buffer, pH 4.8, containing 5 μ l of 30% H₂O₂) for 10 min. The reaction was stopped with 50 μ l per well of 4 M H₂SO₄ and the colour read at 492 nm in a Multiskan Titertek apparatus. Values have been corrected for a PBS blank which was never higher than 0.150. The above conditions were not chosen for optimal sensitivity but for speed and convenience.

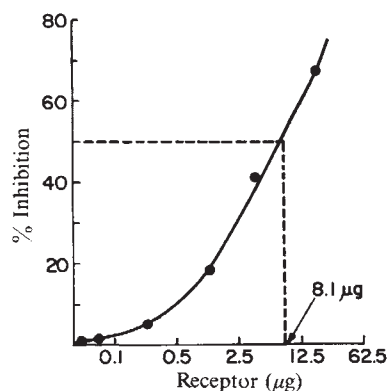


Fig. 2 Inhibition of binding of F8-D5 to anti-BisQ by *Torpedo* AChR. This assay was carried out essentially as described in Fig. 1 legend, except that incubation with F8-D5 and inhibitor (*Torpedo* AChR) was done as follows. Two rows of six wells were used; one served as the control (a blank). PBS-Tween (110 μ l) was added to the first wells of each row; 100 μ l of PBS-Tween were added to the other wells. Then 15 μ l of a purified *Torpedo* AChR preparation (1.52 mg ml⁻¹) were added to the first well of each row. Fivefold serial dilutions were then made by transferring 25 μ l from the first well to the second, 25 μ l from the second to the third, and so on. Finally, 5- μ l portions of F8-D5 (2 μ g ml⁻¹) were added to one row of wells and 5- μ l of PBS to the other row, which served as a control for each of the concentrations of AChR used as inhibitor. Wells containing PBS only and PBS+F8-D5 were also set up. Incubation of the solutions was for 2 h at 37°C. The rest of the assay was done as described in Fig. 1 legend.

of the binding of F8-D5 to rabbit anti-BisQ. These results reinforce the conclusion that the specificity of F8-D5 is for determinants intimately associated with the combining sites of both the *Torpedo* receptor and rabbit anti-BisQ. With respect to the latter, this is consistent with a specificity for an idiotype determinant. It is important to note that BisQ is a small molecule (molecular weight 486) and not likely to block reaction with non-idiotype determinants near the combining site as a result of steric interference.

We have not yet studied the binding of F8-D5 with idiotype determinants of the various monoclonal mouse anti-BisQ antibodies detected in our screening assay (Table 1). However, as F8-D5 arose in mice by immunizing with BisQ-BSA, rather than with rabbit anti-BisQ, it can be concluded that an auto-anti-idiotype immune response occurred. Thus, it seems that idiotype determinants are shared by rabbit and mouse anti-BisQ. We are now carrying out similar quantitative studies with four other clones that have been found to bind both anti-BisQ and receptor.

The strategy described here provides a powerful route to anti-receptor antibodies which are likely to be directed at determinants associated with the combining sites of the receptor. As in our previous findings, purified receptor is not required for immunization; in fact, these anti-idiotype antibodies, pre-

sumably, can be used to isolate receptor. We are in the process of testing this assumption.

Our experimental results also lend support to a functioning idiotype network as foreseen by Jerne⁷. Our earlier studies, in particular the finding of a transient anti-BisQ response when immunizing with anti-BisQ, supported the network theory, as did the report of Schechter *et al.*¹² that immunization with insulin caused the spontaneous appearance of insulin receptor-specific antibodies. Similarly, auto-anti-idiotype antibody appeared during a normal human immune response to tetanus toxoid¹³. Our present studies add to these findings in that in the initial screening of the hybridomas, the number of wells scoring positive for anti-idiotype antibodies was as much as one-half the number positive for anti-antigen antibodies. Thus, not only is the idiotype network functioning during a normal immunization process¹⁴, it is functioning very actively, as early as 5 days after a first booster injection. It should be appreciated that the ease with which we have detected auto-anti-idiotype antibodies depends on the use of hybridoma technology. By immortalizing the clones active at the time of cell harvest, the cellular events are 'frozen' in time, making it possible to study clones that may have only a transient existence *in vivo*. Moreover, as the immortalized clones are separated from each other, this approach avoids the technical problems that arise from the formation of immune complexes and which are an important limitation of serological studies.

Another biological implication of our findings relates to the origins of autoimmune diseases in which anti-receptor antibodies are implicated. At least some of these diseases^{1,12} might originate from an anti-idiotype response to antibodies formed against biologically active ligands normally present *in vivo*, such as insulin or thyrotropin. The likelihood of an anti-idiotype etiology increases if the patients' antibodies are found to be directed at the combining site of a receptor. Although not always the case¹⁵, this has been found to be true in many patients with myasthenia gravis¹⁶, particularly in those who are severely ill. Moreover, we have shown¹ that experimental myasthenia gravis can be induced in rabbits via the anti-idiotype route. Thus, it could have a role in at least some cases of myasthenia gravis in humans. On the other hand, in Graves disease, the specificity of circulating anti-thyroid receptor antibodies is usually directed at the combining site of the thyrotropin receptor¹⁷, so these antibodies are probably anti-idiotype, directed at idiotypes of anti-thyrotropin antibody.

In the earlier studies^{1,12}, only low titres of circulating anti-receptor antibodies were detected and, in our case¹, the anti-idiotype response appeared to be transient. Our present findings show that the anti-idiotype response is, on the contrary, a very important aspect of the response to an antigen. In normal conditions, it must be modulated in some way. If regulation is defective, there could be pathological consequences.

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Table 1 Screening of hybridoma cells

Specificity	Positive wells
BisQ-RSA	14.0% (67/480)
Anti-BisQ	7.4% (55/741)
AChR*	2.4% (22/933)

Numbers in parentheses represent numbers of positive wells per no. of wells assayed. Anti-BisQ is the specifically purified rabbit antibody used in ref. 1.

* Wells positive for AChR were also positive for anti-BisQ, that is, they were a subset of the wells positive for anti-BisQ activity. Purified *Torpedo californica* and rat muscle receptor preparations gave the same results.

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Cell lines producing human T-cell lymphoma virus show altered HLA expression

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Human T-cell leukaemia/lymphoma virus (HTLV) can be identified in fresh and cultured T-lymphocytes from patients with adult T-cell malignancies¹⁻⁶. HLA typing of the peripheral blood lymphocytes and cultured cell lines from the patient from which the virus was originally isolated suggested the expression of additional HLA-A and -B locus antigens on the HTLV positive cultured T-cells that were not present on the EBV transformed B-cell line or on the peripheral blood lymphocytes⁴. Peripheral blood lymphocytes (PBLs) and T-cell lines established from patients and cord blood lymphocytes, infected with virus by co-culture with T-cell lines, were typed for HLA antigens with antisera and in addition tested for reactivity with a monoclonal antibody (4D12) which recognizes a polymorphic HLA class-I antigen⁷. In all HTLV positive cells, with demonstrable provirus replication, altered HLA alloantigen expression was observed. This may be explained by the observations reported in the accompanying paper⁸ which shows homology between the envelope gene region of HTLV and the region of an HLA-B locus gene which codes for the extracellular portion of a class I histocompatibility antigen.

HTLV is a type-C retrovirus and productive HTLV infection can be identified with antisera that has specificity for the p19 and p24 core proteins and reverse transcriptase activity⁹⁻¹¹. The presence of the provirus is detected by nucleic acid hybridization. Cell lines were established from patients' lymphoid cells in liquid suspension using T-cell growth factor (TCGF)⁵. These cell lines were used to productively infect CBL.

Cells from the following sources were used in this study: patients' peripheral blood lymphocytes, cultured cell lines established from the PBLs, cultured cord blood lymphocytes (established with phytohaemagglutinin (PHA) and TCGF), cord blood lymphocytes both infected with HTLV and established by co-culture with non-infected T-cell lines (8402 and HSB-2)^{12,13}, and normal PBLs. HLA typing was performed by the microcytotoxicity technique described by Amos and Pool¹⁴ using alloantisera detecting 16 allelic antigens of the HLA-A locus, 26 alloantigens of the HLA-B locus and 6 alloantigens of the HLA-C locus. In addition to typing with HLA alloantisera, a monoclonal antibody (4D12), which detects a polymorphic HLA determinant, was used. This monoclonal antibody was developed (by B.F.H.) by immunizing mice with a T-lymphoid cell line HUT78. The monoclonal antibody derived from this immunization precipitates molecules of 44,000 and 12,000 molecular weight characteristic of the HLA polypeptides⁷. It is important to note that the precipitation studies were carried out using the HUT78 cell line as well as HUT102, the cell line from which HTLV was originally isolated. Haynes *et al.*

found that 4D12 reacts with normal PBLs that shared the HLA-B5 cross-reactive group of antigens (B15, BW35, BW51, BW52, BW61, and BW62). They also found limited reactions with cells not bearing HLA-B5 antigens. Our studies indicate that in addition to the above antigens this antibody reacts with cells that have specificities including A11, A24, and the A19 cross-reactive group (w29, w30, w31, w32). Reaction of this monoclonal antibody with the cells was tested for using the fluorescence activated cell sorter (FACS) (Becton-Dickinson). The methods of cell labelling and analysis are reported elsewhere¹⁵. All cells showing a forward light scatter pattern were analysed for fluorescence. The percentage of cells reacting with the monoclonal antibody as well as the mean fluorescence was determined, the latter as a measure of the relative density of the antigenic determinant(s) on the cell surface. The P3 mouse myeloma protein was used as a control in these experiments.

Antisera to the p19 and p24 viral core proteins were used to test for productive infection of cells. These proteins cannot be detected on the surface of infected cells. Table 1 shows the HLA phenotypes and 4D12 reactivity of PBLs and cell lines established from patients with HTLV positive leukaemia/lymphoma. The phenotype was assigned based on reactions with at least two sera detecting a particular alloantigenic determinant. In comparing results of the HLA typing of PBLs with cell lines established from PBLs, all cell lines except SD could be assigned more than the two expected HLA alloantigens controlled by the B locus. In one instance (WA cultured) an additional A locus antigen was assigned. The C locus antigen expression was unchanged in culture. The reactivity of the 4D12 monoclonal antibody with these cell lines and the mean fluorescence are also shown in this table. The percentage positive was lower in PBLs compared with cultured cells. Increase in mean fluorescence of the cell population was also found in the cultured cells. Thus, changes in alloantigen expression were observed when changes in the density of the 4D12 antigen occurred. Changes in cell-surface antigen paralleled the pattern of expression of viral core protein. The observations suggest that viral replication may result in the expression of cell-surface antigen that appears to share an antigen determinant(s) with some HLA alloantigens.

Umbilical cord blood lymphocytes were infected with HTLV by co-culturing with irradiated or mitomycin-C treated T-cell lines derived from HTLV positive patients^{5,16,17}. Infection was determined by showing p19 and p24 production. Control cultures were established with PHA and propagated with TCGF. The results of HLA phenotyping and 4D12 reactivity are shown in Table 2. The uninfected cord blood lymphocyte cultures expressed no more than two alloantigens of the HLA-A, B or C loci. The paired infected cell lines typed for the same antigens as the uninfected cells, with the exception of C91/PL, which also expressed what appear to be new HLA determinants. The additional antigens detected could not be because of contamination with the infecting cell line as the HLA phenotypes were the same as those of uninfected cells and the karyotypes of the cell lines were those expected for the cord blood lymphocytes⁵.

The 4D12 antibody reacted with uninfected cell lines when one of the HLA-B5 cross-reactive antigens was detected with alloantisera (C5, C7, C19) and was unreactive with C6 and C8. The latter two cell lines became positive with infection. In all cases productive HTLV infection was associated with increased density of the antigen on the cells as shown by an increase in the mean fluorescence. The results demonstrate that altered HLA antigen expression occurs when cord blood lymphocytes are productively infected with HTLV. The fact that both infected and control cord blood lymphocytes are in tissue culture rules out the possibility that increased reactivity of alloantisera and the monoclonal 4D12 antibody occurred as a result of cell culture. All infected CBL expressed the p19 and p24 core proteins indicating viral replication. Although these data do not show a direct effect of the provirus reproduction on HLA expression, an association of these two events can be inferred.

Table 1 HLA phenotypes and 4D12 reactivity of HTLV positive adult T-cells

Source of cells	HTLV core protein*	HLA phenotype			4D12 reactivity	
		A locus	B locus	C locus	% Positive	Mean fluorescence units
MJ (PBL)	—	1	8,27	w6, w7	8	53
MJ (cultured)	+	1	8,27(17)†	w6, w7	19	346
WA (PBL)	—	1,24	8, w22	w3, w7	80	160
WA (cultured)	+	1,24 (11)	8, w22 (15)	w3, w7	92	1,642
PL (PBL)	—	3, w29	7, w24	w2	36	62
PL (cultured)	+	3, w29	7, w42 (40, w35)	w2	64	385
MI (PBL)	—	28, w33	w45	w2	30	88
MI (cultured)	+	28, w33	w45 (w22, w35)	w2	64	972
OB (PBL)	—	1, w32	44, w49	w7	63	623
OB (cultured)	+	1, w32	44, w49 (w35)	w7	98	1,888
SD (PBL)	—	2, w31	w51, w62	w4, w6	68	787
SD (cultured)	+	w, w31	w51, w62	w4, w6	95	5,642

* Viral core protein p19 and p24 detected by assays described^{9,10}.

† Extra HLA reactions detected in cultured cells.

Table 2 HLA alloantigen expression and 4D12 reactivity on cord blood lymphocytes before and after infection with HTLV

Cell line	HLA phenotype			4D12 reactivity	
	A locus	B locus	C locus	% Positive	Mean fluorescence units
C5 (uninfected)	w32	5, w35	w4	73	760
C5/MJ (infected)	w32, (w30)*	5, w35 (w21)*	w4	82	1,816
C6 (uninfected)	1, w32	40	w2	0.8	14
C6/WA (infected)	1, w32	40 (15)*(w35)*	w2	93	1,688
C7 (uninfected)	2	12, 15	w3	31	244
C7/TK (infected)	2 (w28)*	12, 15 (w22)*	w3	94	800
C8 (uninfected)	3	12	w4	0.5	12
C8/SK (infected)	3 (w31)*(w30)*	12(15)*	w4, w6	80	1,512
C91 (uninfected)	3	w51, w52	w4, w6	80	203
C91/PL (infected)	3	w51, w52	w4, w6	82	1,743

* () Determinants not detected on the cultured non-infected cord blood lymphocytes.

† Infection status verified by *in vitro* expression of viral specific core protein antigens.**Table 3** HLA phenotypes and 4D12 reactivity with CBL co-cultured with HTLV negative T-cell lines and normal PBLs

Cultured cell	HLA phenotype			4D12 reactivity	
	A locus	B locus	C locus	%	Mean fluorescence
C54/8402	2	7, 44	w3	0.1	2
C47/HSB	3, 11	27, w35	—	62	331
C5450/DM	1	w44	w4	7	17

8402 is a T-cell line with an HLA phenotype of A1, Aw19, B5, Bw38. HSB is a T-cell line with an HLA phenotype of A1, A2, B17, B12, Cw2. HLA phenotype DM PBL is A1, A2, Bw35, B40.

The argument that culture conditions are not responsible for altered HLA alloantigen and 4D12 expression is strengthened by results shown in Table 3. As co-culturing is essentially an allogenic stimulus, control cultured cord blood lymphocytes were established with a normal PBL and two HTLV negative T-cell lines HSB-2 and 8402. No additional antigens were detected with alloantisera in these cultures. The 4D12 antigen was not detected in two cultures but was present in a culture where the HLA-BW35 alloantigen was expressed. The mean fluorescence in this culture was similar to that seen in the non-infected cord blood lymphocyte cultures (stimulated by PHA) that were positive for this antigen. The lack of changes in reactivity of alloantisera and the 4D12 antibody with uninfected cultured cells indicates that the observation is not due to an epiphenomenon of *in vitro* tissue culture or some other trivial factor.

The data demonstrate altered expression of HLA or HLA-like antigens associated with productive HTLV infection. While

there are several possible explanations for these observations, the accompanying paper strongly suggests⁸ the antigen(s) detected is a viral protein(s) that bears HLA-like determinants which appear at the lymphocyte surface when the provirus is transcribed. We cannot, however, rule out expression of 'silent' HLA genes and an HLA-like molecule with the epitope common to several alloantigens expressed as a result of integration of the virus in the major histocompatibility complex (MHC) region.

The role of the MHC in tumorigenesis and microbial immunity has been extensively discussed, particularly in murine systems¹⁸⁻²⁰. We now demonstrate potential involvement of human MHC determinants resulting from infection by a human virus that appears to be oncogenic. Certain individuals in HTLV endemic areas have been exposed to or actively carry the virus yet have no demonstrable tumours²¹. The lack of recognition and development of an immune response to antigens that appear as 'self' MHC antigens may result in uncontrolled viral replication and tumorigenesis.

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Homology of human T-cell leukaemia virus envelope gene with class I HLA gene

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Human T-cell leukaemia virus (HTLV), first isolated in the United States from a patient with cutaneous T-cell lymphoma¹, is a unique^{2,3} horizontally transmitted^{2,4} retrovirus which is highly associated with certain adult T-cell malignancies⁵⁻¹¹. Also, HTLV can be transmitted *in vitro* to cord blood T-lymphocytes⁶. In the accompanying paper¹² it was shown that all T cells producing HTLV, whether cultured from infected persons or infected *in vitro*, bind a monoclonal antibody (4D12)¹³ which recognizes an epitope shared by certain cross-reactive class I major histocompatibility antigens. This antigen may account for the extra HLA-A and -B specificities detected in HTLV-infected cells using alloantisera⁴. Because of the unusual findings of apparently inappropriate HLA antigens in HTLV infected cells, we had previously looked for rearrangement of class I-related genes in HTLV infected cells but failed to find any¹⁴. Here, using molecular clones of HTLV and human major histocompatibility antigen DNA, we have shown homology between the envelope gene region of HTLV and the region of an HLA-B locus gene which codes for the extracellular portion of a class I histocompatibility antigen.

Southern blots were prepared from restriction digests of a recombinant λ phage clone of the HTLV provirus. This phage (λ 23-3) contains an 8.2 kilobase (kb) insert of one complete HTLV provirus excised from the integrated state from DNA of the MT-2 cell line¹⁰ with *Sst*I and cloned into the *Sst*I site of λ wes λ B. *Sst*I cuts only within the long terminal repeats (LTRs), and the insert thus contains a provirus which is complete except that it contains only one LTR, and which contains no cellular flanking sequences. We mapped λ 23-3 with restriction endonucleases. The map, presented in Fig. 1, shows that this HTLV clone is indistinguishable from that of Yoshida *et al.*¹¹. A more detailed description of λ 23-3 will be presented elsewhere (E.P.G. *et al.*, in preparation).

The insert from a cDNA clone representing HLA-B7 mRNA cloned into PBR322 (pDPO-1)¹⁵ was excised with *Pst*I, nick translated¹⁶, and tested for homology to HTLV. As shown in Fig. 1, the pDPO-1 insert specifically labelled the HTLV insert in the phage DNA. pBR322 clones of the class II antigens DR- α ¹⁷ and DC-B¹⁸, in contrast, did not label the HTLV insert. Using a combination of double and triple restriction endonuclease digests, the area of strongest homology was mapped to a 600 base pair (bp) region bounded by an *Xho*I site at the 5' end and an *Sst*II site at the 3' end (Fig. 2). A lesser degree of hybridization was observed to the 600 bp *Xho*I-*Xho*I fragment to the 5' side of the *Xho*I-*Sst*II fragment, and to the 1.6 kbp *Sst*II-*Sst*I fragment to the 3' side. The sequences which are

homologous to HLA sequences most likely map within the *env* region of the HTLV genome (Fig. 3).

HLA class I antigens are transmembrane cellular proteins, as is the retroviral envelope glycoprotein. The pDPO-1 clone contains sequences coding for the extracellular, transmembrane and cytoplasmic portions of the HLA protein molecule as well as 3' regions which are not translated. By cleaving the *Pst*I insert of pDPO-1 with *Pvu*II and separating the resultant fragments by gel electrophoresis followed by recovery onto DEAE membrane (Schleicher and Schuell), we obtained sub-fragments which contained different regions and used them as probes for Southern blots of DNA digests from another HTLV proviral clone, λ 372-3 (E.P.G. *et al.*, manuscript in preparation). This clone was digested with the restriction enzymes *Eco*RI and *Hind*III to give a 4.2 kb band containing the *env* region. As shown in Fig. 2, the 5' pDPO-1 *Pst*I-*Pvu*II fragment is 596 bp and represents coding regions for the carboxy-terminal half of the first extracellular domain of the HLA peptide chain, all of the second domain, and the amino-terminal half of the third domain. The internal *Pvu*II-*Pvu*II fragment represents the coding regions for the remainder of the third domain as well as the transmembrane and cytoplasmic domains, while the 3' *Pvu*II-*Pst*I fragment represents the 3' untranslated region. The 5' fragment labelled the HTLV DNA with the same pattern and intensity as the total pDPO-1 probe (Fig. 3), whereas only faint labelling was obtained with the other two probes. Thus, the strongest homology of HTLV *env* sequences is with sequences coding for the extracellular portion of an HLA class I antigen. This was confirmed by a Southern blot with a *Pvu*II-*Pst*I digest of pDPO-1 using the *Clal*-*Hind*III viral fragment of λ 23-3 as a probe (not shown).

To obtain an estimate of the degree of homology between the HTLV *env* and pDPO-1 we washed filters of HTLV DNA hybridized with ³²P-pDPO-1 probe using two different stringencies. Relatively strong signals were obtained when the filters were washed extensively with 2 $\times$ SSC at 50°C. The signal intensities were diminished markedly when the washing temperature was raised to 65°C. From the conditions of hybridization, the salt concentration, and the %G+C content of the pDPO-1 clone, we were able to estimate the base mismatching between the homologous regions of HLA and HTLV using the method of Howley *et al.*¹⁹. Since most of the hybrid appears to melt between 50 and 65°C, there is about a 30-35% mismatch between the two regions. Although we cannot exclude anomalous hybridization to a region of the *env* gene very high in G+C content, there is no such region in the pDPO-1 clone and we have not observed anomalous hybridization when HTLV *env* regions themselves are used as a probe.

We have shown the existence of nucleotide sequence homology between a human retrovirus (HTLV) and a human HLA class I gene and mapped this homology to the viral *env* gene and the coding regions for the extracellular domains of the HLA antigen. It has been reported^{4,12} that cultured HTLV-infected cells apparently express extra HLA-A and -B locus specificities, as well as an antigen detected by a monoclonal

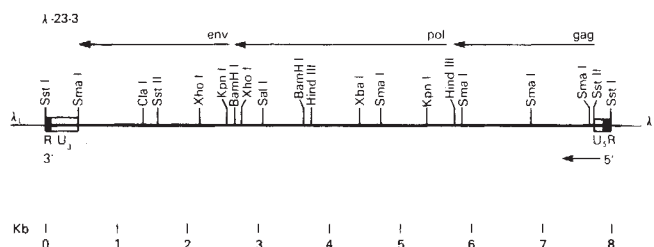


Fig. 1 Restriction endonuclease map of HTLV clone λ 23-3. Clone λ 23-3 was selected from a λ wes λ B library prepared from *Sst*I-digested DNA from the MT-2 cell line using labelled pBR322 clones of the HTLV LTR²³ as probe. The clone was mapped with restriction endonuclease as described previously²⁴. The map is oriented with respect to the left and right λ arms.

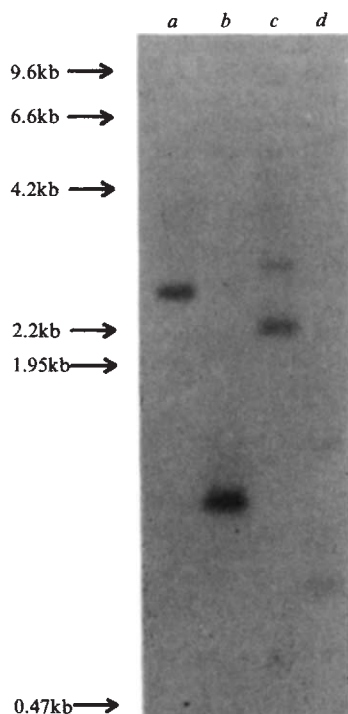


Fig. 2 Homology of HTLV *env* gene to a cDNA clone of HLA class I mRNA. Southern blots of λ 23-3 DNA were performed using 32 P-pDPO-1 as a probe: *a*, *Sst*I and *Bam*HI digestion in which the 2.7 kb fragment containing the HTLV envelope gene and 3' LTR is labelled. *b*, *Sst*I, *Bam*HI and *Sst*II digestion in which a 1.1 kb fragment corresponding to the presumptive 5' region of the HTLV envelope gene is labelled. *c*, *Sst*I, *Xho*I digestion in which a 2.2 kb fragment containing the HTLV envelope gene and 3' LTR is labelled. The 2.8 kb fragment which labels is the result of a partial *Xho*I digestion. *d*, *Sst*I, *Xho*I, *Sst*II digestion in which the 600 bp HTLV envelope gene fragment and 1.6 kb fragment containing the remainder of the envelope gene and 3' LTR are labelled. Arrows indicate the position and sizes of the *Hind*III fragments of λ 23-3 DNA. 1 μ g of λ 23-3 DNA was digested for 2 h with the indicated restriction enzyme in the buffer recommended by the supplier (BRL). The DNA was then electrophoresed through 1% agarose and transferred to nitrocellulose using the techniques of Southern²⁵. Hybridizations were performed as described previously²⁴, using 1×10^6 c.p.m. per ml of pDPO-1 nick-translated to a specific activity of 2×10^8 c.p.m. per μ g. Filters were washed extensively in $2 \times$ SSC, 0.1% SDS at 50 °C.

antibody (4D12) which detects an epitope shared by certain HLA alloantigens¹³. The hybridization data suggest that the appearance of the antigen detected by 4D12 in HTLV-infected cells could be due to expression of the viral *env* gene. Further credence is lent to this notion by the parallel time course of appearance of viral *gag* antigens and the antigen detected by 4D12 when T cells from infected persons are grown in short-term culture (ref. 12 and V. S. Kalyanaraman *et al.*, manuscript in preparation). The expression by these cells of apparent extra HLA antigens may be analogous to the expression of alien H-2 antigens previously reported for murine lymphomas²⁰. Perhaps there is a similar homology to that between HTLV and HLA antigens and H-2 genes and some murine retroviruses. We are currently investigating this possibility.

There are several reasons that suggest the data have biological relevance. If the *env* protein of HTLV were related antigenically to certain HLA antigens, HTLV might use these HLA antigen(s) as a viral receptor. Viral binding would be facilitated to those cells which recognize the homologous HLA antigen. There is some precedence for this type of interaction, as specific binding to HLA and H-2 class I antigens has been reported for Semliki Forest virus²¹. Class I-dependent cell interactions include killing of virus-infected cells. It is possible that

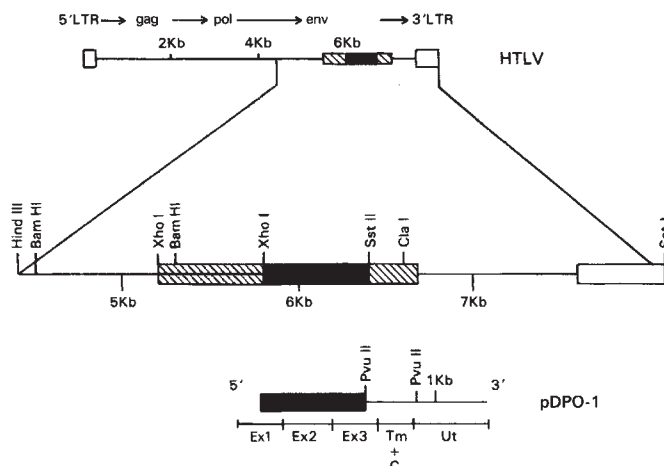
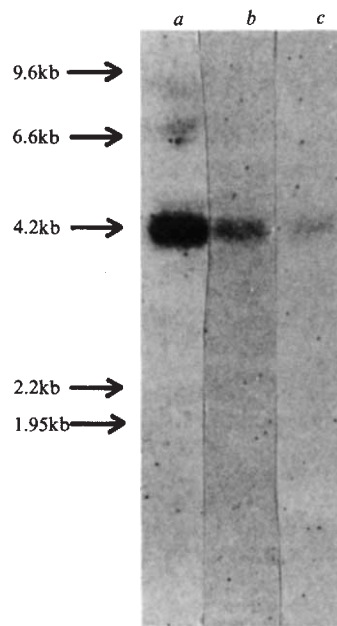


Fig. 3 Regions of homology between clones HTLV DNA and cDNA from an HLA class I mRNA. The areas of strongest homology are represented by dark bars. Hatched bars show the areas giving faint bands when probed with 32 P-pDPO-1. Open bars represent the LTRs. The top map shows the entire HTLV provirus. The coding regions for *gag*, *pol* and *env* are approximate, and are placed by analogy to other retroviruses. The part of the provirus containing the region of homology is expanded to the same scale as the HLA class I cDNA, with the restriction sites used in mapping indicated on both. The domains represented by the different areas of the HLA clone are also indicated. Ex 1, 2, and 3 are the first, second, and third extracellular domains, TM is the transmembrane region, C is the cytoplasmic region, and UT is the 3' untranslated region.

Fig. 4 Localization of homology with HTLV to the region of an HLA mRNA encoding the extracellular domains. Southern blots were performed after an *Eco*RI and *Hind*III digestion of λ 372-3. The probe used in lane *a* is the 5' *Pvu*II fragment of pDPO 1; the probe used in lane *b* is the middle *Pvu*II fragment of pDPO-1; and that used in lane *c* is the 3' *Pvu*II fragment of pDPO-1. Hybridization is to a 4.2 kb fragment containing the HTLV envelope gene and 3' LTR, as well as 3' flanking cellular sequences. Arrows indicate the position and sizes of *Hind*III fragments of λ 372-3. Restriction endonuclease digestions of λ 372-3, electrophoresis, Southern blotting, hybridization and washing were performed as described in Fig. 1, except nick-translated probes were prepared from the indicated fragment of a *Pst*I-*Pvu*II digestion of pDPO-1 recovered from an agarose gel as described in the text.



expression of inappropriate HLA antigens on infected T cells would impair or abrogate their normal function. This might constitute a possible mechanism for the induction by a retrovirus of leukaemia or other immune disorders. The host response to such a phenomenon may be important. Expression of a viral HLA-related antigen that appears as self might allow HTLV-infected cells to evade the cytotoxic T-cell response that would occur if this antigen were recognized as 'non-self'. Finally, there is evidence that major histocompatibility genes can readily undergo homologous recombination or gene conversion²².

Perhaps relatedness to an HLA gene could give HTLV or its *env* gene a preference for certain sites of integration or re-integration into the host cell genome.

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β -Spectrin limits α -spectrin assembly on membranes following synthesis in a chicken erythroid cell lysate

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Spectrin, the major protein of the subcortical actin network in erythrocytes, contains two non-identical subunits, α and β (refs 1, 2). Spectrin is indirectly associated with the transmembrane anion transporter through the binding of β -spectrin to an extrinsic protein, ankyrin³⁻⁵. In chicken embryo erythroid cells, α -spectrin is synthesized in a threefold excess relative to β -spectrin, although the two subunits are assembled in equimolar amounts⁷. To investigate the regulation of assembly of equimolar amounts of spectrin, an *in vitro* system from chicken embryo erythroid cells has now been developed where synthesis and assembly of spectrin can be uncoupled and studied separately. Following the *in vitro* translation of threefold more α - than β -spectrin, 95% of the β -spectrin and equimolar amounts of α -spectrin bind post-translationally to spectrin-depleted rabbit red blood cell membranes, and the excess α -spectrin remains unbound. This α -spectrin cannot bind spectrin-depleted plasma membranes subsequently added to the lysate. The assembly of α -spectrin is, therefore, limited by the availability of β -spectrin, and both subunits assemble post-translationally.

To analyse the *in vitro* synthesis and assembly of spectrin, lysates were prepared from chicken embryo erythroid cells instead of rabbit reticulocytes because we found that the former system synthesizes about fivefold more spectrin at saturating levels of mRNA, and because the chicken system is less susceptible to inhibition of protein synthesis by membranes added

exogenously. As this is the initial report of chicken embryo erythroid cell lysates, we first demonstrated that these lysates synthesize a similar pattern of polypeptides (Fig. 1B) to those synthesized *in vivo* (Fig. 1A). Following *in vitro* translation, immunoprecipitation with antiserum specific for α -spectrin⁸ or β -spectrin⁹ reveals that these lysates (Fig. 1C, lanes b, d) mimic spectrin synthesis *in vivo* (Fig. 1C, lanes a, c) in that about threefold more α - than β -spectrin is synthesized.

We next investigated whether spectrin synthesized *in vitro* was competent to bind to plasma membranes either co- or post-translationally. As only small amounts of spectrin bind to plasma membranes from chicken erythroid cells (Fig. 2, lane b), which cannot be readily depleted of spectrin¹⁰, we prepared spectrin-depleted inverted vesicles from rabbit erythrocytes^{11,12}. Inverted vesicles were then incubated co- and post-translationally with the chicken erythroid cell lysate which synthesized ³⁵S-methionine-labelled spectrin. After incubation, the lysate was separated into a pelleted membrane fraction and a post-membrane supernatant and aliquots were subjected directly to gel electrophoresis. On either co- or post-translational incubation of inverted vesicles in the lysate, α - and β -spectrin are the major translation products to associate with the inverted vesicles (Fig. 2, lanes d, f). Strikingly, while almost all of the β -spectrin and equimolar amounts of α -spectrin associate with the membrane pellet, the excess α -spectrin remains in the post-membrane supernatant (Fig. 2, lanes c, e).

The association of equimolar amounts of α - and β -spectrin

Table 1 Controls on spectrin binding

Expt 1	
Membrane orientation	β -Spectrin pelleted (%)
Inverted vesicles	77
Right-side-out vesicles	4
Inverted vesicles and exogenous spectrin	41
Expt 2	
Inverted vesicle treatment	β -Spectrin pelleted (%)
Untreated vesicles	53
Acetic acid wash	4
Trypsin digestion	1
No membrane	3

In expt 1, fresh inverted vesicles and right-side-out vesicles were prepared from rabbit erythrocytes as previously described^{11,12} and incubated post-translationally (30 min, 30 °C) in the erythroid cell lysate at final membrane protein concentrations of 200 and 280 μ g ml⁻¹, respectively. Crude spectrin (90% α - and β -spectrin by electrophoresis) was extracted from rabbit erythrocyte plasma membranes in a small volume of 0.1 mM NaPO₄ at 37 °C (ref. 11), and added at a final protein concentration of ~20 μ g ml⁻¹ to inverted vesicles (200 μ g ml⁻¹ membrane protein) during incubation in the lysate. Following incubation, membranes were pelleted at 12,500g for 15 min. The supernatant and solubilized membranes were then assayed by immunoprecipitation for α - and β -spectrin. As β -spectrin is the limiting subunit in assembly, for clarity and to avoid correcting for the 3:1 ratio of α : β -spectrin synthesis, results are expressed in terms of percentage of β -spectrin pelleted. This value was obtained by densitometry of fluorographs of immunoprecipitates, and dividing the peak area of β -spectrin in the pellet by the combined peak areas of the supernatant and pellet. Equimolar amounts of α -spectrin pelleted with inverted vesicles in the presence of exogenous spectrin, and similar decreases in spectrin binding were obtained with a second preparation of inverted vesicles (data not shown). In expt 2, fresh inverted vesicles were washed with 0.1 M acetic acid for 30 min (0 °C) then with buffer¹¹, or digested with 1 μ g ml⁻¹ trypsin for 20 min (20 °C) before addition of 1.3 mM phenylmethylsulphonyl fluoride. Treated and untreated membranes were pelleted at 150,000g for 15 min in a Beckman airfuge before post-translational incubation in the erythroid cell lysate at similar membrane concentrations to those in expt 1. After spectrin binding for 30 min (30 °C), membranes were again pelleted at 150,000g before quantification of spectrin as in expt 1.

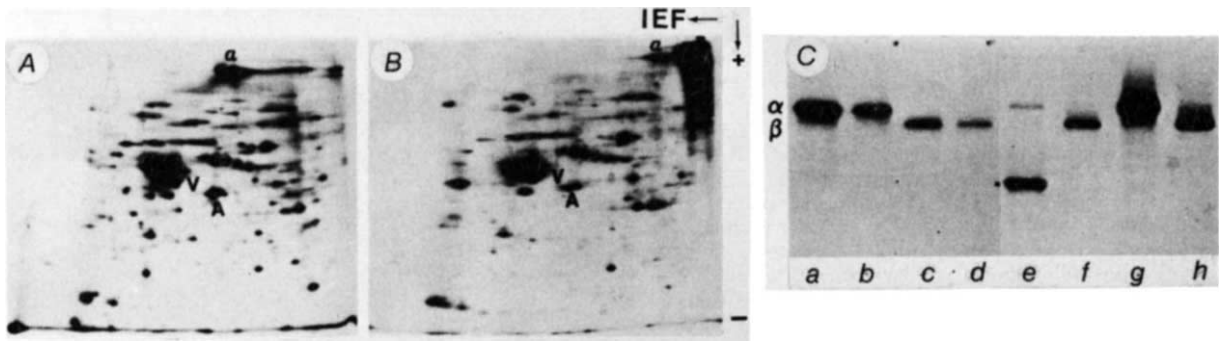


Fig. 1 Comparison of chicken erythrocyte polypeptides synthesized *in vivo* and *in vitro*. **A**, To analyse polypeptides synthesized *in vivo*, erythrocyte cells from 15-day-old chicken embryos were washed, labelled with ^{35}S -methionine, solubilized and subjected to isoelectric focusing⁸, SDS 10% polyacrylamide gel electrophoresis⁷ and fluorography⁷. α -Spectrin (α), vimentin (V) and actin (A) are noted on the fluorograph. The fluorographs were exposed for 24 h in **A**, and 96 h in **B**. **B**, Polypeptides synthesized *in vitro* were separated on gels as above. The preparation of the *in vitro* translation system from chicken erythrocyte cells uses methods similar to those applied to rabbit reticulocytes⁷. Erythrocyte cells from 10–15-day-old chicken embryos are obtained by removing embryos from extraembryonic membranes, and bleeding isolated embryos into Petri dishes containing Eagle's balanced salt solution (20 °C). The erythrocyte cells are then filtered through four layers of cheesecloth, collected by centrifugation in a clinical centrifuge, and resuspended in ice-cold 134 mM NaCl, 5 mM KCl, 7.5 mM MgOAc, 5 mM glucose, 10 mM HEPES, pH 7.4 at 22 °C. The cells are then washed four to six times by repeated centrifugation in this buffer at 0 °C, with the removal of any buffy coat after each centrifugation. The lysate is prepared by adding 1.5 volumes of glass distilled water (0 °C) to the pelleted cells. As these cells do not lyse as readily as rabbit reticulocytes, hypotonic lysis is achieved by gently mixing the suspension with a pipette for 5 min. The lysate is then centrifuged in a Sorvall HB4 rotor at 18,000g for 15 min (0 °C) followed by freezing and storing aliquots of the supernatant under liquid nitrogen. Smaller volumes of lysate can also be prepared by centrifugation in 1.5-ml microcentrifuge tubes. Standard translation reactions, using components described elsewhere¹⁶, contain 480 μl of lysate, 10 μl of 1 mM haemin (Sigma) in 90% ethylene glycol, 10 μl of 5 mg ml⁻¹ creatine kinase (Boehringer) in 50% glycerol, 30 μl of 2 M KCl, 10 mM MgCl₂, 30 μl of 0.2 M creatine phosphate (Boehringer), 30 μl of a solution containing 2 mM of all amino acids (Calbiochem-Behring) except methionine, adjusted to pH 7.4 with KOH and supplemented with 1 mM dithiothreitol, 8 μl of 0.2 M EGTA, adjusted to pH 7.4 with KOH, 12 μl of 4 mg ml⁻¹ total chicken erythrocyte cell RNA prepared as previously described⁷, 30–60 μl of ^{35}S -methionine, aqueous, ~1 mCi ml⁻¹, 1,200 Ci mmol⁻¹ (Amersham or NEN). Lysates are incubated at 30 °C for 120 min except where noted. Kinetics of incorporation are linear for up to 2 h, with generally 350,000 c.p.m. of trichloroacetic acid-insoluble ^{35}S being incorporated per 2- μl aliquot of lysate after 90 min incubation. Translational activity of the lysate without added RNA was variable, and up to 10-fold less than the lysate supplemented with mRNA. All lysates were therefore supplemented with total RNA from erythrocyte cells of comparably staged embryos. Lysates supplemented with homologous RNA then reproducibly displayed high translational activities. **C**, Synthesis of α - and β -spectrin *in vivo* and *in vitro*. Immunoprecipitation (method B⁷) with antibodies specific for α -spectrin⁸ or β -spectrin⁷, SDS 10% polyacrylamide gel electrophoresis and fluorography were used to compare the relative mobilities and subunit ratios of ^{35}S -methionine-labelled α -spectrin synthesized *in vivo* in erythrocyte cells from 15-day embryos (**a**), and *in vitro* in the cell-free lysate (**b**), and β -spectrin synthesized *in vivo* (**c**) and *in vitro* (**d**). In separate experiments the effect of EGTA in preventing degradation was examined by performing *in vitro* translations in the absence (**e**, **f**) or presence (**g**, **h**) of 2.5 mM EGTA followed by immunoprecipitation of α -spectrin (**e**, **g**) and β -spectrin (**f**, **h**) from the translation mixtures. The discrete degradation product of α -spectrin is denoted by a $\bullet$ in **e**. Leupeptin at 1 mM similarly prevented the degradation of α -spectrin, but the total yield of ^{35}S -methionine-labelled α - and β -spectrin was less than that obtained with EGTA (not shown). β -spectrin exhibits no detectable degradation in the presence or absence of EGTA. Only the top portion of the gel is shown in **C**. Entire gels of immunoprecipitated spectrins are shown in Fig. 2. The fluorographs in **C** were exposed for 9–19 h. The apparent unequal synthesis of α - and β -spectrin is not an artefact of *in vitro* translation because an approximately 3:1 ratio of α - to β -spectrin is also synthesized *in vivo*⁷. This ratio is also not an artefact of immunoprecipitation because spectrin synthesized in rabbit reticulocyte lysates⁷ or chicken erythrocyte cell lysates (Fig. 2, for example, lanes **a**, **k**), and examined by gel electrophoresis of total translation products instead of immunoprecipitation, also reveal the excess synthesis of α - relative to β -spectrin. An approximately 3:1 ratio of α - to β -spectrin is also obtained by immunoprecipitation using serially diluted samples or samples boiled in SDS (data not shown), which establishes the independence of the subunit ratio from sample size used for immunoprecipitation, and precludes any effect of spectrin aggregation on subunit ratio. Unequal turnover also cannot account for the unequal subunit ratio because the 3:1 ratio is obtained by short labelling periods *in vivo*⁷. Furthermore, examination of *in vitro* translation products obtained at 5-min intervals during a 90-min translation, and at 0 and 30 min after terminating translation, confirm the synthesis of excess α -spectrin.

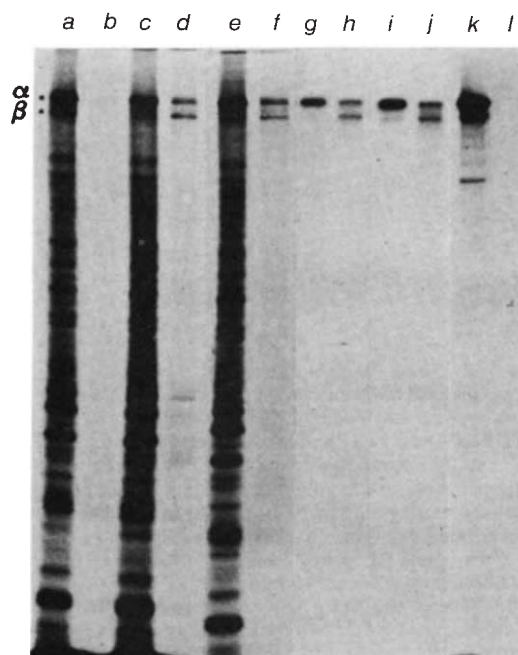
with inverted vesicles, from a pool containing a threefold excess of α - relative to β -spectrin in the lysate, was further demonstrated by immunoprecipitation of spectrins from the membrane and supernatant fractions of the inverted vesicles incubated in the lysate (Fig. 2). As was evident from examining the total ^{35}S -methionine-labelled polypeptides, immunoprecipitation and quantification of spectrins revealed that ~95% of the β -spectrin and equimolar amounts of α -spectrin associate with inverted vesicles (Fig. 2, lanes **h**, **j**). The α -spectrin which is in molar excess to the bound β -spectrin does not associate with the membrane and is recovered in the post-membrane supernatant (Fig. 2, lanes **g**, **i**).

Several types of experiment suggest that spectrins synthesized *in vitro* bind to a finite number of sites on the cytoplasmic surface of the plasma membrane. The specific requirement for binding to the cytoplasmic surface of membranes is demonstrated by the absence of pelleted spectrin in lysates incubated

without membranes (Fig. 2, lane **l**, and Table 1), and by the negligible amount of spectrin binding to chicken erythrocyte membranes not depleted of spectrin (Fig. 2, lane **b**), or to right-side-out rabbit erythrocyte vesicles (Table 1). The requirement of protein sites for spectrin binding to the plasma membrane is suggested by data showing that a brief treatment of inverted vesicles with acetic acid or trypsin¹¹ abolishes spectrin binding (Table 1). A more specific control shows that 50% inhibition of β -spectrin binding is achieved by adding 20 $\mu\text{g ml}^{-1}$ of exogenous rabbit spectrin to lysates incubated with 200 $\mu\text{g ml}^{-1}$ inverted vesicles (Table 1). Similar to previous data on the reassociation of extracted erythrocyte spectrin with inverted vesicles^{11,12}, in the present study the amount of labelled spectrin in the membrane pellet increased linearly with increasing concentrations of membranes within the range of 75–300 $\mu\text{g ml}^{-1}$ membrane protein, and was time dependent (data not shown). Finally, because equimolar amounts of α - and

Fig. 2 Stoichiometric binding of avian α - and β -spectrin to mammalian inverted erythrocyte vesicles. *a*, Total ^{35}S -methionine-labelled polypeptides of a post-membrane supernatant co-translationally supplemented with chicken erythroid cell membranes ($250\ \mu\text{g ml}^{-1}$ membrane protein, prepared as previously described¹⁰). *b*, Total ^{35}S -methionine-labelled polypeptides associated with the pelleted chicken erythroid cell membranes. *c*, *e*, Total post-membrane supernatants of lysates supplemented co-translationally (*c*) or post-translationally (*e*) with rabbit erythrocyte inverted vesicles ($225\ \mu\text{g ml}^{-1}$ membrane protein). *d*, *f*, Total ^{35}S -methionine-labelled polypeptides associated co-translationally (*d*) or post-translationally (*f*) with pelleted rabbit inverted vesicles. The α - and β -spectrin in similar samples from a different experiment were measured by immunoprecipitation. Quantification by densitometry of the fluorographs of the α - and β -spectrin present in the co-translational supernatant (*g*) and membrane pellet (*h*), and the post-translational supernatant (*i*) and membrane pellet (*j*), indicate that the spectrin-depleted inverted vesicles bind 95% of the β -spectrin, and equimolar amounts of α -spectrin, and that the excess α -spectrin remains in the post-membrane supernatant. The presence of both spectrin subunits in the supernatant of a larger aliquot of lysate (*k*) incubated without membrane, and the absence of spectrin in the corresponding 'pellet' (*l*) illustrate the membrane dependence of spectrin sedimentation to the pellet. The fluorograph of the SDS 10% polyacrylamide gel, which contained 5% sucrose in the separating gel, was exposed for 6 h.

Methods: Erythrocyte vesicles which were both inverted and partially spectrin-depleted were prepared from adult rabbit erythrocytes^{11,12} and suspended in membrane buffer (25 mM NaCl, 1 mM KCl, 1.5 mM MgOAc, 1 mM glucose, 2 mM HEPES, 0.5 mM dithiothreitol, 50 μM EGTA, pH 7.4) to a concentration of $2.2\ \text{mg ml}^{-1}$ membrane protein. Protein concentrations were estimated according to Bradford¹⁷. For co-translational spectrin binding, 10 μl of membrane suspension were added per 100 μl of chicken erythroid lysate and translation was conducted for 60 min at 30°C . For post-translational spectrin binding, 40 μM emetine was added to lysates after 60 min of translation, membrane suspensions were added to 10% (v/v), and the binding conducted for 30 min at 30°C . Inhibition of protein synthesis by emetine was determined by measuring trichloroacetic acid-insoluble ^{35}S . Following co- or post-translational spectrin binding, the lysates were separated into pelleted membrane and soluble fractions by centrifugation at $12,500g$ for 15 min. Pelleted membranes were suspended in a buffer containing Triton X-100⁷ to the same volume as the supernatant and then crystalline urea and β -mercaptoethanol were added to 9 M and 2.5%, respectively. Equal aliquots were used for immunoprecipitation as in Fig. 1 except that the α - and β -spectrin antibodies were first mixed together. The stoichiometric co- and post-translational association of the spectrin subunits with the inverted vesicles cannot be attributed to the dilution of newly synthesized and ^{35}S -methionine-labelled spectrins into soluble pools of unlabelled spectrins. We have directly measured by immunoprecipitation the ratios of α - and β -spectrin in the small soluble spectrin pool in the lysate and observed an α - to β -spectrin ratio of approximately 3:1 (data not shown). As this is the same ratio as that observed for newly synthesized chicken erythroid spectrins *in vivo* and *in vitro*, the only effect of the unlabelled spectrin pool would be to lower the specific activity of the labelled spectrin available for binding to membranes.



β -spectrin bind to the inverted vesicles from a protein-rich lysate, whereas the excess α -spectrin and numerous other translation products do not, it is apparent that there is great selectivity in the binding of equimolar amounts of spectrin subunits to inverted vesicles in this cell-free system.

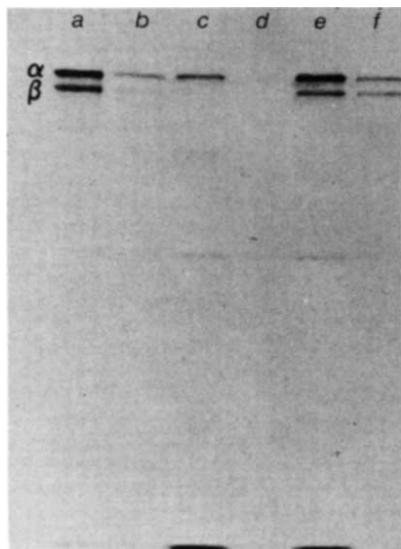
While all β -spectrin and equimolar amounts of α -spectrin can be bound to membranes in the lysate at membrane protein concentrations of $225\ \mu\text{g ml}^{-1}$ (Fig. 2), two-thirds of the α -spectrin remains unbound, suggesting that β -spectrin limits the binding of α -spectrin to plasma membranes. This hypothesis was further investigated *in vitro* by first titrating all labelled β -spectrin, and equimolar amounts of labelled α -spectrin, onto saturating amounts of inverted vesicles ($1,500\ \mu\text{g ml}^{-1}$ membrane protein) which were then pelleted to remove the labelled bound spectrin (Fig. 3, lane *a*). The supernatant, containing labelled α -spectrin and no detectable β -spectrin, was then incubated with fresh inverted spectrin-depleted vesicles ($86\ \mu\text{g ml}^{-1}$ membrane protein) for 30 min. The membranes were subsequently pelleted and analysed for α -spectrin by immunoprecipitation. The labelled α -spectrin was found exclusively in the post-membrane supernatant (Fig. 3, lane *c*), with no α -spectrin detected in the inverted vesicle membrane pellet (lane *d*), which is consistent with the above hypothesis. As a control on this procedure, lysates incubated with right-side-out vesicles ($1,900\ \mu\text{g ml}^{-1}$ membrane protein) bound little spectrin (Fig. 3, lane *b*). However, after pelleting these vesicles and mixing the supernatant with inverted vesicles ($86\ \mu\text{g ml}^{-1}$, a subsaturating membrane concentration), β -spectrin and equimolar amounts of α -spectrin still bound to inverted vesicles (Fig. 3, lane *f*). These experiments strongly suggest that β -

spectrin limits α -spectrin assembly.

Previous work established that α -spectrin is synthesized in chicken erythroid cells *in vivo* in a 3:1 ratio relative to β -spectrin, the subunits are assembled in equimolar proportions, and the excess α -spectrin turns over⁷. In the present report we have demonstrated *in vitro* that newly synthesized spectrin assembles on the plasma membrane when incubated co- or post-translationally in a chicken erythroid cell lysate, suggesting that assembly *in vivo* occurs post-translationally. That this post-translational assembly is regulated only by β -spectrin and the β -spectrin binding site was demonstrated by the inability of α -spectrin to bind membranes independently of β -spectrin. These results may also have implications for recently reported non-erythroid forms of spectrin^{8,12-15}. We expect that the expression of non-erythroid β -spectrin-like polypeptides and their receptors will determine spectrin distribution and assembly, independent of the form and quantity of α -spectrin which is expressed. Our approach of using an *in vitro* erythroid cell lysate to study spectrin assembly also suggests that lysates may be supplemented with membranes or mRNAs from any source, thus also allowing studies on the synthesis and the regulation of assembly of non-erythroid spectrins.

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Fig. 3 β -Spectrin limits the assembly of α -spectrin. Spectrin was synthesized *in vitro* in the absence of membrane for 60 min (30 °C), followed by the addition of 40 μ M emetine to stop protein synthesis. Inverted vesicles^{11,12} (at 1.5 mg ml⁻¹ final concentration of membrane protein) or right-side-out vesicles¹¹ (at 1.9 mg ml⁻¹) were added at a concentration sufficient for the inverted vesicles to bind all of the β -spectrin and incubated for 30 min (30 °C). The lysate containing inverted vesicles was then depleted of β -spectrin



and equimolar amounts of α -spectrin by pelleting the membrane (lane a). As a control on the procedure, the right-side-out vesicles which bind little spectrin were also pelleted (lane b). The post-inverted vesicle supernatant from lane a was then incubated with fresh inverted vesicles (86 μ g ml⁻¹ membrane protein) for 30 min (30 °C), and then separated into a post-membrane supernatant (lane c) and membrane pellet (lane d). The post-right-side-out vesicle supernatant from lane b was also incubated for 30 min with inverted vesicles (86 μ g ml⁻¹), then separated into a post-membrane supernatant (lane e) and membrane pellet (lane f). All samples were adjusted to the same volume before immunoprecipitation using mixed α - and β -spectrin antisera. The fluorograph was exposed for 11 h.

nucleus and a symbiotic autonomous prokaryote, is an intriguing unanswered question, although that such genetic exchange is possible is strongly suggested by recent reports of homology between maize mitochondrial and chloroplast genomes³ and the presence of mitochondrial sequences in yeast nuclei⁴. We now present evidence that the spinach nucleus contains integrated sequences that are homologous to ctDNA sequences and that these sequences are incorporated at specific sites within the genome. The experiments also show a region of homology between ctDNA and mtDNA.

There is homology to ctDNA in all spinach tissues (Table 1), ranging from about 980 copies of the ctDNA molecules per haploid genome in the chloroplasts of leaves, down to 60 copies per haploid genome located in the plastids of root cells. In nuclei isolated from roots there is the equivalent of about four copies of ctDNA per haploid spinach genome, which could be due either to a low level of contamination of the isolated nuclei by chloroplast DNA, or to the presence of chloroplast related sequences within the nucleus.

Both nuclear (n) and ctDNA are digested by the restriction endonuclease *EcoRI* (Fig. 1). After separation by agarose gel electrophoresis ctDNA digests (lane 4) show a number of characteristic discrete fragments, while the nDNA (lane 1) has a less defined pattern with several discrete bands, representing reiterated sequences, superimposed on a background of heterogeneous restriction fragments. The restriction endonuclease *HpaII*, a methylation sensitive enzyme⁵, does not digest the bulk of the nDNA (lane 2) but cleaves ctDNA into pieces of less than 4.0 kilobase pairs (kbp) (lane 5).

A ³²P-labelled 7.7 kbp *PstI* cloned fragment of spinach ctDNA was hybridized to restriction fragments from various combinations of nuclear and ctDNA digested with *PstI*, *EcoRI* and *HpaII* (Fig. 1, lanes 6–15). Chloroplast DNA digested with *PstI* yields the expected single hybridization band of 7.7 kbp (lane 6), and three major bands when digested with *EcoRI* (lane 8). The *EcoRI* bands of ctDNA are reduced in size to less than 3.0 kbp in double digests with *HpaII* plus *EcoRI* (lane 11), and the low level of contamination of the nDNA with ctDNA is demonstrated by the lack of these chloroplast-specific bands in lanes 9, 10 and 13. Nuclear DNA digested with *EcoRI* (lane 9) shows major hybridization bands of 8.9, 6.3, 6.1 and 4.7 kbp and some minor bands, none of which correspond to the ctDNA *EcoRI* fragments hybridized with the 7.7 kbp probe, although the bands at 6.3 and 6.1 kbp are close to one chloroplast band (lane 8). In contrast to the ctDNA *EcoRI* hybridization pattern, that of nDNA is unchanged after digestion with *HpaII* plus *EcoRI* (lane 10). Digestion of nDNA by *HpaII* (lane 13) leaves essentially all of the sequences homologous to the probe undigested. Similar results are obtained after *EcoRI* digestion of a mixture of ctDNA and nDNA when the three characteristic ctDNA hybridization bands can be seen superimposed on the nDNA pattern (lane 7). Only these ctDNA bands are further cleaved on digestion with *HpaII* and *EcoRI*, leaving the nDNA *EcoRI* bands undisturbed (lane 12). These experiments, which separate ctDNA from nDNA by organelle isolation and by differential sensitivity to *HpaII*, indicate that the nDNA sequences that are homologous to the 7.7 kbp *PstI* fragment of spinach ctDNA are integrated into the spinach nuclear genome.

The pattern of hybridization of total root DNA to the 7.7 kbp *PstI* fragment is similar to that of nDNA (Fig. 2, lanes 1–5), except for the large ctDNA component in roots (Table 1). The *EcoRI* digest (Fig. 2, lanes 1 and 2) is dominated by heavy hybridization to the ctDNA component, but *HpaII* plus *EcoRI* (lane 3) digests ctDNA to small fragments revealing a set of larger *HpaII*-insensitive fragments similar to those found in nDNA (Fig. 1). The fragments are better defined in root DNA because the initial DNA sample had a higher molecular weight. These results, obtained with total root DNA, confirm the presence of nDNA–ctDNA homologies. In addition to the bands that correlate well with those found in nDNA (Fig. 1), there is another prominent band at 3.9 kbp (see below).

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Sequence homology between spinach nuclear and chloroplast genomes

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Relatively few chloroplast (ct) or mitochondrial (mt) proteins are encoded and synthesized within the organelles, the majority being made on cytoplasmic ribosomes from messenger RNAs transcribed from nuclear genes^{1,2}. How this interaction of genetic systems evolved is poorly understood. Whether the chloroplast or mitochondrial specific genes now residing in the nucleus arose *de novo*, or by genetic exchange between the

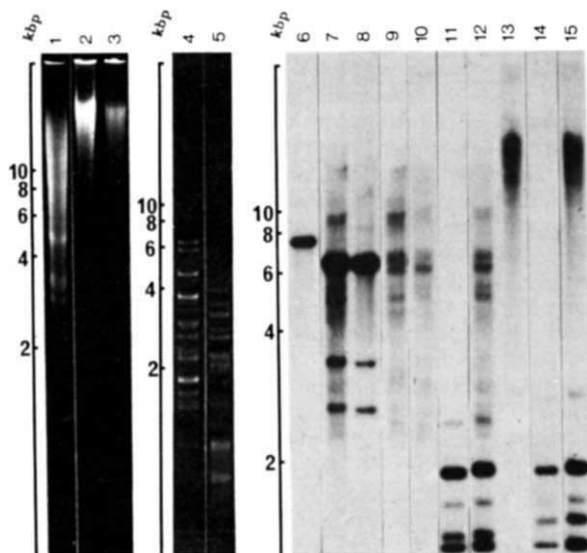


Fig. 1 Detection of sequence homologies between nuclear and ctDNA in spinach with a cloned 7.7 kbp *Pst*I fragment of spinach DNA. Nuclear DNA, digested with *Eco*RI (lane 1), *Hpa*II (lane 2) and undigested (lane 3), and ctDNA digested with *Eco*RI (lane 4) and *Hpa*II (lane 5) was stained with ethidium bromide. The autoradiographs (lanes 6–15) are of hybridizations of the 7.7 kbp *Pst*I fragment 8° of ctDNA cloned in pBR322⁹ to ctDNA digested with *Pst*I (lane 6); nuclear and ctDNA (lane 7), ctDNA (lane 8) and nDNA (lane 9), digested with *Eco*RI; nDNA (lane 10), ctDNA (lane 11) and nuclear and ctDNA (lane 12), digested with *Hpa*II plus *Eco*RI; nDNA (lane 13), ctDNA (lane 14) and nuclear and ctDNA (lane 15), digested with *Hpa*II.

Methods: DNA was digested for 6 h with restriction enzymes (Boehringer Mannheim) as recommended by the manufacturer, except that digestions of nDNA contained 5 units of enzyme per μ g of DNA. In the case of double digests the *Eco*RI digestion was second after suitable adjustment of the buffer. The formaldehyde fixation of tissue, prior to organelle isolation, has no effect on the digestion of nDNA (described here) or ctDNA (unpublished results) by restriction endonucleases. Following electrophoresis on 0.8% agarose gels the DNA was transferred to nitrocellulose filters¹⁷ and the filters hybridized¹⁸ with 2×10^6 c.p.m. per ml of ³²P-labelled probe. The probes were labelled by nick translation¹⁹ with [α -³²P]dCTP to a specific activity of 5×10^8 c.p.m. per μ g. The ctDNA loading (4.0 ng per lane) compared with the nDNA loading (5.0 μ g per lane) is equivalent to six copies of ctDNA per haploid spinach genome. Molecular weights were calculated by comparison with *Hind*III plus *Eco*RI restriction fragments of phage DNA.

The endonuclease *Hpa*II digests DNA at the sequence C/CGG, but not at the methylated sequence C/^mCGG (ref. 5). As there is no detectable methylation of ctDNA⁶ and there are many *Hpa*II sites in ctDNA (Fig. 1, lanes 5, 11), the *Hpa*II-insensitive nuclear fragments homologous to the 7.7 kbp *Pst*I probe must be methylated at any *Hpa*II sites. The *Eco*RI fragments in root DNA hybridizing to the probe are also insensitive to *Msp*I, which cleaves DNA at C/CGG or C/^mCGG but not at ^mC/CGG (Fig. 2, lane 5). Since neither *Hpa*II nor *Msp*I are able to cleave these sequences in nDNA, it seems most likely that the tetranucleotide CCGG in nuclei is doubly methylated (that is, ^mC^mCGG), as seems to be the case in wheat germ DNA where at least 40% of the CCGG sites are unavailable to either *Hpa*II or *Msp*I⁷.

The homologies observed might have been due to mtDNA contamination. Two *Eco*RI fragments of a mtDNA preparation show homology to the 7.7 kbp *Pst*I probe (Fig. 2, lane 7). The minor band (6.1 kbp) is of the same size as the most prominent band in the ctDNA *Eco*RI digests (Fig. 1, lane 8) and probably represents contamination of mtDNA with ctDNA. The major fragment (8.8 kbp), however, does not occur in ctDNA (Fig. 1) and does not coincide with any bands in the nDNA digests, indicating that the nDNA used in these experiments has no

Table 1 Chloroplast DNA levels in spinach DNA preparations

Source of DNA	ctDNA (% of total)	ctDNA (copies per haploid nucleus)
Leaves	21.0	980
Petioles	9.2	470
Leaf initials	7.2	370
Roots	1.5	62
Root nuclei	0.07	4

DNA was isolated¹⁴ from spinach plants grown in liquid culture¹⁵. Nuclei were isolated from roots which had been extensively washed in dilute detergent. The roots were fixed in ice cold 0.5% formaldehyde in 0.01 M HEPES pH 7.0 for 1–2 h, homogenized 4×3 s in a razor blade blender in 0.025 M Tris-HCl pH 7.5, 0.275 M sorbitol, 0.005 M β -mercaptoethanol and 0.5% Triton X-100 and the homogenate filtered through one layer of cheesecloth and three of miracloth. The nuclei were collected by centrifugation at 400g for 5 min, resuspended in the extraction buffer, underlaid with a similar buffer containing 0.4 M sorbitol and collected by centrifugation at 400g for 5 min. This was repeated three times. The amount of ctDNA in the spinach DNA preparations was estimated from the renaturation kinetics of added spinach ³²P-ctDNA¹⁴. The diploid DNA content of the spinach nucleus is 1.9 pg (ref. 16) and spinach ctDNA is 145 kbp in length⁶, allowing the number of copies of the plastome per haploid genome to be calculated.

detectable contamination with mtDNA. Both these bands in mtDNA contain *Hpa*II and *Msp*I sites (Fig. 2, lanes 8, 9). After double digestion of mtDNA by *Eco*RI plus *Hpa*II (lane 8), by *Hpa*II (lane 9), and by *Msp*I (lane 10), a single major hybridization band (3.9 kbp) is generated which is not present in ctDNA. This fragment can also be seen in *Hpa*II and *Msp*I digests of total DNA from roots (lanes 3–6), a tissue rich in mitochondria. The size and intensity of the band in lanes 8–10 suggest that it is a product of the 8.8 kbp band in lane 7, while the small minor bands in lanes 8–10 are characteristic of *Eco*RI and *Hpa*II digests of ctDNA (Fig. 1) and probably result from *Hpa*II digestion of the 6.1 kbp *Eco*RI fragment in lane 7. The homology between this mtDNA sequence and the 7.7 kbp *Pst*I ctDNA fragment, which also has homology with nDNA, is another example of 'promiscuous' DNA^{3,8}, and raises the possibility that there may be DNA sequences common to all three genetic compartments.

Hybridization of further cloned fragments of ctDNA⁹ to chloroplast and nDNA is shown in Fig. 3, lanes 1–6. The *Pst*I fragments of 13.5 kbp and 12.3 kbp flank the 7.7 kbp fragment¹⁰ used in Fig. 1, while the *Xho*I 3.0 kbp fragment originates from the ribosomal (r) RNA gene region of the inverted repeat sequence in ctDNA¹⁰. The results parallel those shown in Fig. 1, with each ctDNA probe hybridizing to a specific set of *Eco*RI fragments in ctDNA, and to a different and more diverse set of fragments in the nDNA digests.

It is possible that the sequence homologies observed are due to similar functional requirements of products derived from both nuclear and chloroplast genes. For example, the *Xho*I 3.0 kbp ctDNA sequence contains part of the 23S rRNA gene¹⁰ and might therefore show some homology with the nuclear rDNA sequences^{11,12}. After hybridization of nDNA with the 3.0 kbp *Xho*I fragment, the probe was removed from the filter, and the nDNA rehybridized with a probe containing cloned spinach nuclear rDNA. Two of the four strong bands produced by hybridization with the *Xho*I 3.0 kbp clone also co-migrate with bands hybridizing to the nuclear rDNA sequence (Fig. 3, compare lanes 7, 8), indicating the possibility of some nDNA-ctDNA homology for this region of the structural RNA gene. The three smaller nDNA *Eco*RI fragments hybridizing to rDNA (lane 8) are predicted by the restriction enzyme map of the cloned nuclear rDNA (unpublished data). The two larger fragments result from partial digestion of the sequence. Scintillation counting of the individual bands in lane 8 indicates that the restriction is 94% complete. It is unlikely, therefore, that the

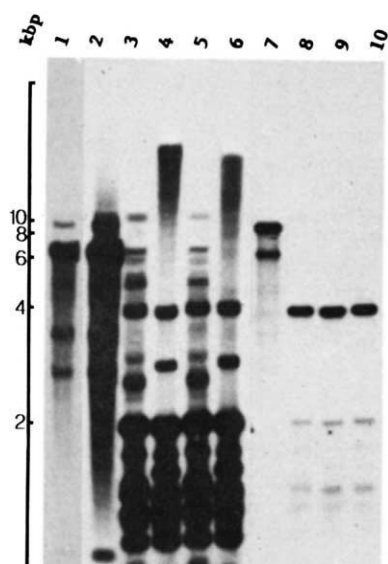


Fig. 2 Detection of sequence homologies in total root DNA and mtDNA with a cloned 7.7 kbp *Pst*I fragment of spinach ctDNA. Root DNA digested with *Eco*RI (lanes 1, 2), *Hpa*II plus *Eco*RI (lane 3), *Hpa*II (lane 4), *Msp*I plus *Eco*RI (lane 5) and *Msp*I (lane 6); and mtDNA digested with *Eco*RI (lane 7), *Hpa*II plus *Eco*RI (lane 8), *Hpa*II (lane 9), *Msp*I (lane 10), were hybridized to the 7.7 kbp *Pst*I fragment of ctDNA. Lane 1 is a shorter exposure of lane 2.

Methods: Mitochondria were isolated²⁰, resuspended in 0.3 M mannitol, 0.001 M EDTA, 0.01 M MgCl₂, 0.03 M MOPS, pH 7.2, and 0.2% bovine serum albumin, and incubated at 0°C for 1 h with DNase (1 mg ml⁻¹) and venom phosphodiesterase (500 µg ml⁻¹). After addition of EDTA to a final concentration of 0.1 M, the mtDNA (0.2 µg per lane) was purified as described for chloroplasts¹⁴. Total root DNA (5 µg per lane) was prepared²¹ from roots grown in liquid culture¹⁵. Samples of DNA were digested and autoradiograms prepared as described in Fig. 1.

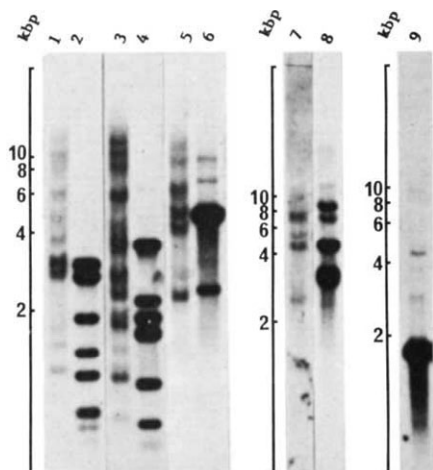


Fig. 3 Detection of sequence homologies between nuclear and ctDNA in spinach. Nuclear DNA (5 µg, lanes 1, 3, 5, 7, 8), ctDNA (4 ng, lanes 2, 4, 6) and root DNA (5 µg, lane 9) were digested with *Eco*RI and hybridized to 13.5 kbp *Pst*I (lanes 1, 2), 12.3 kbp *Pst*I (lanes 3, 4) and 3.0 kbp *Xho*I (lanes 5-7) ctDNA fragments. After removal of the hybrids formed with the 3.0 kbp *Xho*I fragment, lane 7 was hybridized with cloned spinach nuclear rDNA (lane 8). Root DNA was hybridized with the 1.75 kbp *Eco*RI ctDNA fragment (lane 9), containing the gene for the large subunit of RuBP carboxylase.

Methods: Autoradiograms were prepared as described in Fig. 1 legend. The 12.3 and 13.5 kbp *Pst*I fragments¹⁰ were cloned in pBR322⁹, the 1.75 kbp fragment (pSocE48) in pBR325¹³ and the 3.0 kbp *Xho*I fragment in pACYC177⁹. The complete spinach nuclear rRNA gene was cloned in pACYC184 (unpublished results).

ctDNA-nDNA hybridization observed is complicated by partial digestion of the nDNA.

A 1.75 kbp *Eco*RI fragment of ctDNA essentially encompasses the whole of the gene for the large subunit of ribulose biphosphate (RuBP) carboxylase and its flanking sequences¹³, the products of which are encoded and synthesized in the chloroplast. After hybridization of this fragment to root DNA digested with *Eco*RI, at least four nuclear fragments, as well as the expected ctDNA *Eco*RI fragment, are found (Fig. 3, lane 9).

The experiments described show that ctDNA-nDNA homologies are apparent with ctDNA probes which cover about 25% of the plastome. We have used the entire spinach ctDNA clone bank⁹ as hybridization probes to *Eco*RI and *Bam*HI restriction digests of nDNA, and each clone contains sequences that have homologous nuclear counterparts. This is consistent with the experiments from which Table 1 was derived where the extent of renaturation of the labelled ctDNA was similar in the presence of either nuclear (64% renatured) or chloroplast (63%) DNA. By comparing the levels of *Pst*I 7.7 kbp probe hybridization in nDNA gel lanes with those in a calibrated range of ctDNA loadings the amount of ctDNA in nDNA was estimated to be about 3-6 copies of the plastome per haploid genome (compare with Table 1).

The detection of ctDNA related sequences in discrete restriction fragments in nDNA and total root DNA suggests that related sequences are integrated into the nDNA at a number of specific sites. The general lack of correlation of bands in the nuclear and ctDNA restriction patterns homologous to ctDNA indicates that long continuous tracts of homology are rare. On the other hand the two major bands of 2.6 and 2.9 kbp in the *Eco*RI digest of nDNA hybridized to the 13.5 kbp *Pst*I ctDNA fragment (Fig. 3, lane 1) correspond with those in the digest of ctDNA (lane 2), suggesting that this region could contain a longer tract of homology. The number of homologous bands in the nDNA restriction pattern suggests that a particular ctDNA sequence has homology in a small number of different nuclear locations.

These experiments with cloned ctDNA restriction fragments are the first to demonstrate unambiguously the covalent integration of ctDNA-homologous sequences within a plant nucleus. Whether these homologies reflect an archaic or continuing evolutionary process of chloroplast-nuclear sequence transfer; what the direction and molecular mechanisms of transfer are; and whether these homologies are related to the redistribution of genetic functions between cellular compartments, are all questions for further experimentation.

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Targeted mutagenesis of SV40 DNA induced by UV light

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UV light is a potent mutagen in most living cells, but molecular analysis of its mode of action in animal cells has not been possible because of the high complexity of the cell genome. Therefore, we have used simian virus 40 (SV40) as a biological probe¹ to study the mutagenic effect of UV-irradiation at the molecular level. A thermosensitive SV40 mutant of the large T antigen (tsA58), which is defective at high temperature for initiation of viral DNA replication and for repression of early transcription^{2,3}, was UV-irradiated *in vitro*, then replicated in monkey kidney cells. Revertants of tsA58 were selected for growth at the nonpermissive temperature, then the reversion sites were mapped by using the marker rescue technique⁴, and precisely localized by DNA sequencing. We report here that all revertants analysed showed one or two base pair substitutions localized in the C-terminal half of the T antigen gene. The original tsA58 mutation was still present in all revertant genomes. In 16 DNAs sequenced, seven reversion sites were found on the large T antigen gene, all of which localized opposite a possible UV-induced pyrimidine-pyrimidine lesion, suggesting targeted mutagenesis. UV-irradiation of the host cell before infection did not change the pattern of reversion sites at the molecular level, although it strongly increased the mutation frequency⁵. These results demonstrate for the first time in animal cells the specificity of UV-induced mutagenesis.

SV40 is particularly useful in such a study because: (1) it is repaired and replicated as a minichromosome using only host cell enzymes (except that the viral T antigen is necessary for the initiation of SV40 DNA replication); (2) numerous mutants can easily be obtained, mapped and sequenced; (3) the virus can be UV-irradiated *in vitro* and mutagenized in a variety of cells either untreated or treated with various chemicals or physical agents¹.

Our mutation assay was based on the reversion of the SV40 early tsA58 mutant to the wild-type phenotype at the nonpermissive temperature. The spontaneous reversion rate of the tsA58 mutant is low ($\sim 10^{-6}$), while UV-irradiation of the virus strongly increases the mutation frequency in proportion to the degree of irradiation^{5,6}. Moreover, UV-irradiation or carcinogen treatment of the host cell before infecting with UV-irradiated tsA58 increases both virus survival and virus mutagenesis^{5,6} (Table 1). Plaques from SV40 revertants growing at 41 °C, obtained after growth of UV-irradiated tsA58 in control or UV-irradiated monkey kidney cells, were isolated. To ensure that the revertants were really composed of genetically homogeneous virus, plaque assays were carried out at 41 °C at a high dilution to yield only one or two virus plaques per 60-mm Petri dish. The very low spontaneous reversion rate of the tsA58 mutant allowed us to exclude the presence of pre-existing revertants in the original infectious samples, as infections were initiated at a very low multiplicity of infection (10^{-3} PFU per cell).

SV40 DNA was prepared from these revertants and the reversion sites were mapped by the marker rescue technique⁴. Figure 1 shows that the reversion sites which are able to 'rescue' the tsA58 defect, are mapped in either the B, H or I fragment of the *Hind*II+III restriction map. No complementation has been found in the A fragment, which represents the first 1,200 nucleotides of the T antigen gene. In 40% of the revertants, two different DNA fragments of the same genome were able to complement independently the tsA58 defect (the purity of each fragment was carefully checked by using various restriction enzymes). We cannot offer any specific explanation for the presence of two different reversion sites, apart from the fact

that selection pressure is very high and that one can select by chance two independent mutation events.

We have sequenced some of the complementing fragments to localize the mutational sites precisely. Table 2 shows that only one base pair substitution was found for each complementing fragment, except in two cases where two substitutions were found, indicating that one or two base pair substitutions are responsible for the phenotype reversion. All revertants have conserved the original tsA58 mutation site (a G-C to A-T base pair substitution localized in the I fragment which replaces alanine with a valine residue⁶). The fact that several independently isolated revertants mapped at the same DNA sequence sites indicates that the number of possible reversion sites is quite limited on the T antigen gene; this is not surprising as the mutation we have selected for must produce an active thermostable enzyme. Furthermore, in the case of one revertant (R-36-4) base changes occurred at two different sites within the same complementing fragment. One of these changes occurred at the same site as for the other single base change revertants (site 3), suggesting that the second change may be a 'silent' one, that is, may not be relevant to the protein reversion pattern (site 2).

The limited number of sites which have been found renders it difficult to elaborate a general rule for UV-induced mutagenesis in animal cells. However, there are three interesting specificities. First, only base pair substitutions have been found, as is the case in *Escherichia coli*⁷, M13 phage⁸ and yeast⁹. The number of transition sites is roughly identical to the number of transversion sites. Second, the mutation sites are not distributed randomly but are present in all cases in a minimal sequence of T_{A-T}^{T-T} or T_{A-G}^{T-C} . As SV40 DNA is double-stranded, it is difficult to determine which strand is mutagenized first; however, it is tempting to speculate that pyrimidine dimers, which should have been present after UV-irradiation in all mutagenized localizations, are directly responsible for the mutagenesis found. This result is in complete agreement with the mode of mutagenesis of UV light in the *lacI* gene of *E. coli*⁷ and in the *CYC1* locus in yeast⁹. However, this preferential mutagenicity of UV light on sites containing adjacent pyrimidines has not been found in the sequences of various revertants of amber M13 phages produced after UV-irradiation⁸. The fact that M13 phage contains a single-stranded (ss) DNA genome may explain the difference in the mutation pattern, either due to specific secondary structures of ss-DNA or

Table 1 Survival and mutation frequency of UV-irradiated tsA58 SV40 mutants grown in control or UV-irradiated monkey kidney cells

Revertant no.	UV dose (Jm ⁻²) to:		Viral survival (PFU ml ⁻¹) at:		Mutation frequency ($\times 10^{-4}$)
	Cells	Virus	33 °C	41 °C	
R-102-A	0	1,500	3×10^4 *	3	1 ± 0.2
R-59-1					
R-61-1					
R-13-5	5	1,200	350†	59	$1,690 \pm 150$
R-13-7					
R-14-8					
R-14-10					
R-62-1	5	1,500	2.5×10^4 *	100	40
R-63-1					
R-67-1	5	2,000	3×10^3 *	170	560
R-36-4	10	2,000	300†	80	2,670
R-45-4	15	1,500	400†	16	400

CsCl-purified stock viruses of tsA58 mutant were UV-irradiated *in vitro* with a germicidal lamp (mainly at 254 nm). Virus infection occurred for 2 h on either untreated confluent monolayers of CV1-P monkey kidney cells, or cells UV-irradiated 24 h previously⁵. After one lytic cycle, carried out either at 33 °C for 4 days (*) or 37 °C for 3 days (†), cells were frozen and thawed, and progeny survival was measured by plaque assay on CV1-P cells at 33 or 41 °C. The mutation frequency ($\pm$ s.d.) is the ratio of progeny survival at 41 °C to that at 33 °C. Revertant plaques were picked at 41 °C, and stock viruses and DNA were produced.

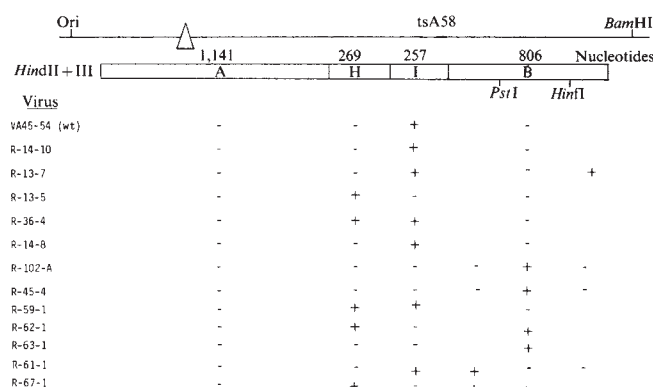


Fig. 1 Localization of the reversion sites of tsA58 on the T antigen gene. The top line represents the early part of the SV40 genome from the origin of replication (Ori) to the *Bam*HI site, with the splice site (Δ) and the tsA58 mutation site indicated. The wild-type parent of tsA58 (VA 45-54) complements the temperature-sensitive mutation on the I fragment as described previously^{4,6}. - Represents no complementation between the isolated fragment and the tsA58 DNA (<5 PFU per 0.1 μ g of purified DNA). + Represents a positive complementation between the isolated fragment and tsA58 mutation (>100 PFU per 0.1 μ g purified DNA). Two (or more) + signs indicate that two (or more) fragments were independently able to complement the tsA58 mutation.

Methods: DNA from revertants, isolated as described in Table 1, were digested with *Hind*II+III restriction enzymes and the 11 principal fragments (A-K) were isolated on 4% polyacrylamide gels. Each fragment from the early region (A, H, I and B) was annealed *in vitro* with form II tsA58 DNA as described by Lai and Nathans⁴. After DNA transfection, plaques were counted directly at 41 °C on CVI-P cells. The absence of contamination during the isolation of DNA fragments was checked carefully by using various restriction enzymes.

Table 2 DNA sequences at mutation sites of various revertants of the tsA58 mutation

Revertant no.	Complementing fragment sequenced	Mutation site	tsA58 sequence	Revertant sequence
R-13-5	H	Site 1 3931	A. T T G. A	A. T C G. A
R-36-4			T. A A C. T	T. A G C. T
R-59-1			gln	arg
R-62-1				
R-36-4	I	Site 2 3596	A. A A A. G	A. A A C. G
			T. T T T. C	T. T T G. C
			phe	val
tsA58	I	3505	A. A A C. A	A. A G C. A
			T. T T G. T	T. T C G. T
			val	ala
R-13-7	I	Site 3 3495	A. T T C. A	A. A T C. A
R-14-8			T. A A G. T	T. T A G. T
R-14-10			glu	asp
R-36-4				
R-61-1				
R-59-1				
R-61-1	B	Site 4 3402	C. A A A. A	C. C A A. A
			G. T T T. T	G. G T T. T
			phe	leu
R-67-1	B	Site 5 3334	T. G T C. C	T. G C C. C
			A. C A G. G	A. C G G. G
			asp	gly
R-45-4	B	Site 6 3180	A. A T C. T	A. C T C. T
R-102-A			T. T A G. A	T. G A G. A
			asp	glu
R-102-A	B	Site 7 2936	G. C C A. A	G. C C G. A
R-67-1			C. G G T. T	C. G G C. T
			trp	arg

DNA sequencing was done by using the Maxam and Gilbert method¹⁹, on all the complementing fragments indicated in Fig. 1. The sites of substitution (tsA58 sequence) and mutation (revertant sequence) are shown in heavy type. In each case, only one base pair substitution was found for each complementing fragment, except for the B fragment of R-102-A and the I fragment of R-36-4. Identical mutation sites were found in several independently isolated revertants. The position of the mutation site is given by the nucleotide number, according to the published SV40 nucleotide sequence²⁰. Arrows indicate the 5'→3' direction of the codon frame. The B fragment of the R-67-1 revertant has two different mutations. Treatment of the B fragment with *Hinf*I and *Pst*I restriction enzymes produces four fragments, two of which are able independently to 'rescue' the tsA58 mutation because of two different reversion sites (sites 5 and 7).

to the specific mode of replication of this phage. Recently, Haseltine *et al.*¹⁰ reported novel UV-induced photoproducts—the pyrimidine(6-4)pyrimidine lesions. These base damages measured along the *lacI* gene of *E. coli* seem to correlate with nonsense mutation hotspots of the same gene¹⁰. As pyrimidine dimers and Py(6-4)Py lesions can be formed in a Py-Py sequence by UV light, although at different frequencies, the exact nature of the mutagenic lesion could not be known at that time¹⁰.

Finally, in bacteria, the induction of SOS repair functions is necessary to give rise to mutation both at DNA lesions and in undamaged DNA^{11,12}. With animal viruses, various authors have found that DNA lesions are sufficient to produce mutation even in non-induced cells^{5,6,13-16}, although pretreatment of cells before infection with damaged DNA virus strongly increases the mutation frequency^{5,6,13}. In our experiment, the type of mutation was the same in UV-irradiated cells and control cells (Tables 1 and 2); only the mutation frequency was significantly increased.

To initiate SV40 DNA replication, the T antigen must bind to specific DNA sequences at the origin of replication. The T antigen from the tsA58 SV40 mutant is unable to bind to regions around the SV40 DNA initiation site at the nonpermissive temperature, at least *in vitro*^{2,17}. Each reversion site that we have localized is able to restore this binding activity of T antigen protein. It is unknown why the tsA58 T antigen cannot bind to SV40 DNA initiation sites and why revertant T antigens become able to do so. Either DNA-protein interactions or protein-protein interactions are involved in the thermosensitivity of the T antigen. By computer analysis, we have determined the secondary structure of wild-type, tsA58 and revertant T antigens according to Garnier *et al.*¹⁸. The tsA58 mutation is localized in the middle of a 10-amino acid α -helix. Revertants at site 3 (three amino acids from the tsA58 site; Table 2) do have around the tsA58 site a shorter α -helix of only five amino acids due to the presence of a new β -helix ending at the tsA58 site. The other sites are mapped either in an α -helix zone or in a β -helix in the tsA58 mutant which is usually shortened or interrupted in the revertants (P. Dessen and A. S., unpublished data). The exact significance of these secondary structure changes is still unknown, but they clearly indicate that only one base pair substitution in a 708-amino acid protein is sufficient to change locally the secondary structure at specific sites. However, definitive predictions cannot be made on enzyme-DNA interactions, as the rules of such interactions and the tertiary structure of the T antigen are not yet known.

During these experiments, we analysed by polyacrylamide gel electrophoresis the *HinfI*, *HindII* and *HindIII* restriction maps of at least 40 DNA revertants; we found no detectable deletion or addition in any of them. However, we found by chance in one revertant (R-61-1) a new *HindIII* restriction site in the middle of the late genes at nucleotide 1,585. The DNA sequence data of this region indicated that one base pair substitution had transformed the original sequence (AAGCTC) into the mutant AAGCTT, producing a novel *HindIII* restriction site, while the amino acid sequence of the VP₃ protein was unchanged. Again in this case, for which no specific selection was used, we found a single base pair substitution opposite a possible pyrimidine dimer site or a G-T(6-4)C lesion. In the same vein, we determined the DNA sequence of the early splice site for four revertants; no DNA base changes were recorded for 1,400 nucleotides sequenced. This result indicates that either base changes can be lethal inside the splicing region or the probability of base changes for any unselected position is <1/1,400.

These results suggest that in animal cells probed with SV40 DNA, pyrimidine-pyrimidine DNA lesions induced by UV light are mutagenic *per se* through a base pair substitution pathway. Although SOS mutation activity has not been completely demonstrated in animal cells, one can hypothesize that UV-induced mutations occur through a mis-replication process of pyrimidine dimers or pyrimidine(6-4)pyrimidine lesions,

indicating that targeted mutagenesis is the most probable event after UV irradiation in animal cells.

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Determination of twist and handedness of a 39-base pair segment of DNA in solution

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For DNA in solution, there have been two methods for measuring the helical periodicity. One method^{1,2} examines the ladder of discrete bands formed in the electrophoretic pattern of supercoiled DNA. Such a ladder is assumed to reflect the set of differently linked covalently closed molecules. The other method^{3,4} measures the lengths of partial nuclease digestions of DNA segments affixed to flat surfaces. Neither method, however, is able to measure the absolute handedness. X-ray diffraction of DNA oligonucleotides can determine the handedness⁵⁻⁷, but the DNA must necessarily be crystallized. We have developed a new method for determining the helical parameters of a segment of DNA in solution. It is the first non-crystallographic method that can directly determine both the helical periodicity and the absolute handedness of DNA. The results obtained using this method are consistent with the classical Watson-Crick right-handed double helical model⁸.

We first applied our method to the analysis of the twist and handedness of a 39-base pair (bp) linear segment of DNA in solution. (Our use of the terms 'twist' and 'linking number' follows the terminology of Crick⁹.) The scheme of the method is outlined in Fig. 1. We took advantage of the fact that the single-stranded phage DNA of the pair of cloning vectors M13mp8 and M13mp9 developed by Messing¹⁰, are complementary through a 39-bp stretch (the cloning region). There is a unique *AccI* restriction endonuclease recognition site close to the centre of the cloning region. We cleaved the replicative form of M13mp9 with the endonuclease *AccI* and hybridized it to excess M13mp8 phage DNA, allowing the formation of the two major hybridization products. One of these products consists of two single-stranded DNA pieces hybridized only

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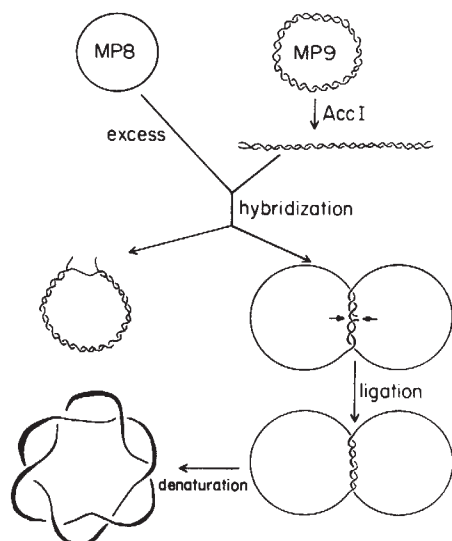


Fig. 1 Schematic outline of the reactions used in the determination of the twist and handedness of the 39-bp segment of DNA. This outline assumes the right-handed double helical model, and so the final denatured structure consists of two single-stranded closed curves that are right-handedly linked. A left-handed model would have predicted left-handedly linked structures following the denaturation step.

through the 39-bp complementary region. Ligation of this product with T4 DNA ligase should then result in two covalently closed circles linked together by a value equal to a near integer value of the twist of the 39-bp hybrid region. Since the linking number of the complex must remain invariant as long as neither covalently closed circle is broken, we reasoned that denaturation of the 39-bp hybrid region should sufficiently separate the hybrid region for subsequent measurement of the linking number by electron microscopy. Because the linking number must remain invariant under deformations of the two linked circles as long as neither one is broken, the linking number can be measured without regard for the possible changes caused by spreading procedures during the preparation of samples for microscopy. As for the handedness, it is merely the polarity of the linking number and thus measurable for the same reasons. It followed that, if the 39-bp hybrid region were originally a right-handed helix, the two denatured linked circles must also be wound in a right-handed orientation. We carried out the above reactions (outlined in Fig. 1 and detailed in Fig. 2 legend) and indeed observed many complexes of two linked single-stranded circles as shown in Figs 2 and 3. To determine the linking pattern of two linked circles, the contour and shadow patterns were used in conjunction with the fact that the contour lengths of each single-stranded ring had to remain equal to each other and equal to the contour length of an unlinked M13 single-stranded circle. The approximate relative numbers of clearly linked molecules with 1, 2, 3 and 4 links between the two circles were 14, 17, 42 and 23 respectively. No molecules with a linking number greater than 4 could be observed unequivocally. Some structures with high linking numbers may have been dismissed as too complex and ambiguous for an assignment of a linking number. The distribution of molecules with different linking numbers presumably reflects various fluctuations during the ligation reaction.

It is important to note that we were careful to preserve the handedness of the original specimen throughout all of the processes required to produce the final image, from the placement of the grid into the microscope to the printing of the final photograph¹¹. In each structure, the overall handedness of the two linked rings, otherwise known as the polarity of the linking number, was determined by examining each junction where one strand of one ring crossed over a strand of the other ring. In each of the linked structures we examined, most of the

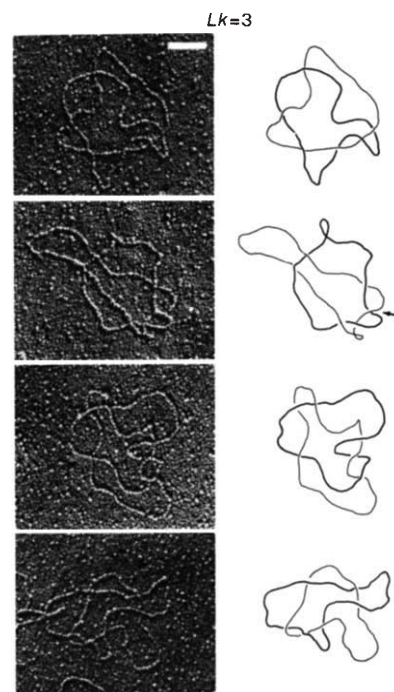


Fig. 2 Electron micrographs of two single-stranded right-handedly linked rings formed following the reactions outlined in Fig. 1. The linking number of each of these structures is 3 ($Lk = 3$). Line drawing interpretations are shown beside each electron micrograph. The arrow indicates a cross-over junction at which one of the DNA strands has happened to fall in a line parallel to the direction of shadowing. In such an instance, the orientation of the cross-over is ambiguous (see text). Scale bar, 0.2 μm . 1 μg of M13mp9 RF was digested with *AccI* restriction endonuclease until all of the DNA was linearized. After phenol extraction and ethanol precipitation, the linearized DNA was dissolved in 30 μl of water to which 15 μl of 1 M NaOH were added. After a 10-min incubation, 60 μl of 50 $\text{ng } \mu\text{l}^{-1}$ M13mp8 single-stranded circular DNA were added, followed by 15 μl of 2 M Tris-HCl and 3.6 μl of 5 M NaCl. Hybridization of the mixture was for 5 min at 65 $^{\circ}\text{C}$ followed by 40 min at 55 $^{\circ}\text{C}$. The DNA was then ethanol-precipitated and ligated. The final ligation was at 15 $^{\circ}\text{C}$ for 1 h in a reaction volume of 50 μl and 400 units of T4 DNA ligase (New England Biolabs). The ligation reaction was quenched with EDTA, and the DNA was phenol-extracted and ethanol-precipitated. The ligated sample was denatured in 98% formamide at 65 $^{\circ}\text{C}$ for 5 min, adjusted to concentrations of 92% formamide, 50 mM Tris (pH 8.5), 5 mM EDTA, 10 $\mu\text{g ml}^{-1}$ of cytochrome *c* and then immediately spread onto a hypophase of 62% formamide, 5 mM Tris (pH 8.5) and 0.5 mM EDTA. The DNA was picked up with a parlodion-coated grid 4–5 min after spreading and shadowed once with rotation and once at a fixed angle. The handedness of the original specimen was preserved in the micrographs as described in ref. 11, and the procedure checked by observing the handedness of supercoils in simian virus 40 DNA with and without the addition of ethidium bromide.

cross-over junctions were consistent with those expected of a right-handedly linked set of two rings (see Fig. 1 for the convention of right-handed linkage). In a linked structure, if a cross-over junction containing one strand happened to lie parallel to the direction of the shadowing of the electron microscope grid, then the cross-over orientation was obscured. If we then ignored those few cross-over junctions where one of the DNA strands happened to have fallen parallel to the shadowing direction, all of the linked molecules appeared to possess cross-overs expected of a right-handedly linked structure. In Fig. 2, for example, there are several molecules each of which possesses right-hand junctions at four or five of the six cross-over junctions. The ambiguous cross-over junctions are always cases when a DNA strand has fallen in a line parallel to the direction of shadowing. Since in each case, the two circles are definitely

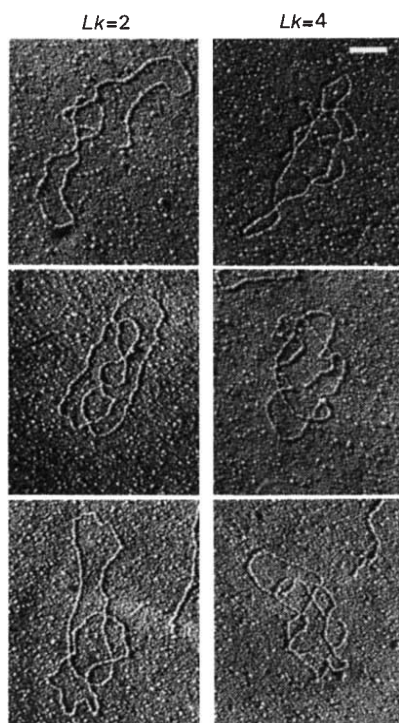


Fig. 3 Electron micrographs of two interlocked single-stranded rings produced following the reactions outlined in Fig. 1. The structures in this figure are linked twice ($Lk = 2$) or four times ($Lk = 4$). Scale bar, 0.2 μm .

linked, they must be linked either right-handedly or left-handedly. The fact that we did observe many clearly right-handed junctions in each linked structure, led us to conclude that the overall handedness was indeed of the right-handed orientation. Our results, therefore, were totally consistent with the Watson-Crick right-handed double helical model.

That the two circles were indeed linked following the reactions outlined in Fig. 1, was also demonstrated by alkaline agarose gel electrophoresis. As shown in Fig. 4, molecules with approximately twice the molecular weight of M13 phage DNA circles were observed only after the final ligation reaction in the series of steps outlined in Fig. 1. We are now developing gel electrophoresis techniques to determine the linking number of two interlocked single-stranded circles.

The handedness of the DNA duplex region was also ascertained by another method using two M13 single-stranded DNA circles containing cloned complementary sequences. The hybridization of the two circles forms a duplex region between the complementary sequences. Due to the helical nature of the duplex region, a degree of twist is produced in the duplex segment which must be countered by an equal but oppositely oriented winding of the single-stranded portions of the circles around each other. Using two pairs of M13 phage clones containing complementary sequences of approximately 200 and 1,000 bp, we examined the hybrid structures and observed that the windings of the noncomplementary single-stranded DNA were left-handed. This observation was yet another non-crystallographic means of determining the absolute right-handedness of DNA. The number of windings of the single-stranded parts of the circle around each other could not be determined by electron microscopy because of the spreading procedure's effect on the conformation of DNA in the duplex region.

We conclude that our method shows that the 39-bp segment of DNA we studied is indeed of a right-handed double helical conformation in solution. The limitation of this method lies in the difficulty of counting the linking number of two highly linked circles using electron microscopy. We have measured the twist to be around 3 or 4 which is not far from the value expected based on the helical periodicity of random DNA measured by

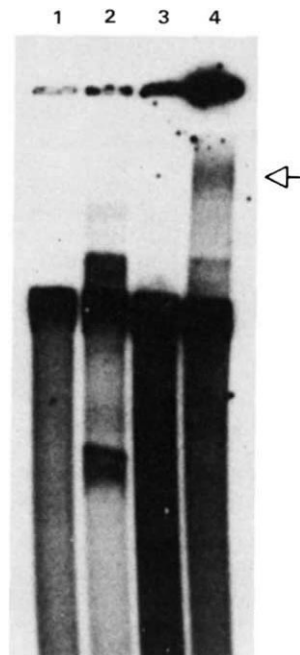


Fig. 4 Alkaline agarose gel electrophoretic analysis of DNA samples showing the formation of linked circles. 1 μg of M13mp9 RF was linearized with *AccI*. After phenol extraction and ethanol precipitation, the DNA was resuspended in 5 mM ADP, 50 mM imidazole-Cl (pH 6.6), 10 mM MgCl_2 , 5 mM dithiothreitol, 0.1 mM spermidine, 320 μCi of $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ (3,000 Ci mmol^{-1} ; NEN) and 20 units of T4 polynucleotide kinase. After a 30 min incubation at 37 $^\circ\text{C}$, the reaction was terminated with EDTA. The DNA was then phenol-extracted and ethanol-precipitated. 187 ng of the kinase-treated DNA was used to carry out all of the reactions outlined in Fig. 1 and described in Fig. 2 legend. The final product after the ligation was run on lane 4. Another 187 ng of the reaction product omitting the ligation step were run on lane 3; 62 ng of the kinase-treated linear DNA were run on lane 1 and 62 ng of the kinase-treated linear DNA incubated with DNA ligase were run on lane 2. Each of the DNA samples was first incubated in 0.3 M NaOH for 10 min before loading on a 0.8% agarose gel in a 30 mM NaOH, 10 mM EDTA buffer. The gel was run for 11 h at 200 mA, then dried and exposed on Kodak XAR5 for 16 h. The arrow points to the band of lane 4 which represents the linked circles. (The radioactive counts at the origin of the gel in lane 4 represent complicated side products of the hybridization reaction.)

other means^{1,2,4}. The determination of more precise values for the helical periodicity must await the development of other physical techniques such as gel electrophoresis. The convenient multiple restriction endonuclease recognition sites within the cloning region of M13 cloning vectors allow almost any linear DNA segment to be inserted. The method described here is thus potentially applicable to measuring DNA conformational changes as a result of DNA-protein or DNA-drug interactions, or even sequence-dependent alterations, within a well-defined region of DNA sequence.

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Origin of granitoid rocks

CLAYBURN *ET AL.*¹ have recently stated that "Moorbath² has presented Pb-Sr isotopic evidence that a mantle component is present in granitic rocks of all ages, a feature which can be considered indicative of continuous primary continental accretion". This is an unintentional partial misrepresentation of my views on this subject, which have also been misquoted in other recent papers. This confusion arises because Clayburn *et al.*, as well as many other workers, tend to use and interpret the broad, all-encompassing term 'granitoid' interchangeably with the specific term 'granitic', thereby frequently lumping together rock units of totally different petrogenetic lineages, for which there is no justification in most of the published data from isotope geology or experimental petrology³⁻⁶.

Published isotopic evidence demonstrates that true granites of any geological age in continental tectonic environments frequently have a significant or major component derived from older sialic crust. Indeed, many granites (s.s.) may be the products of pure crustal anatexis (see ref. 7). In contrast, calc-alkaline granitoids (such as diorite, tonalite, granodiorite) of any geological age—and especially voluminous orthogneisses of the major Precambrian shield areas—appear to be predominantly derived from basic lithosphere with mantle-like geochemical and isotopic characteristics, although in some tectonic environments there may be isotopic and other evidence for significant crustal contamination of mantle-derived magmas (see ref. 8).

In this connection, the view of Allègre and Othman⁹ (quoted by Clayburn *et al.*¹) that granitoids younger than ~2,000 Myr contain predominantly recycled crustal component but that older ones are juvenile mantle extracts makes no petrogenetic, tectonic or other distinction between true granites and calc-alkaline, intermediate rocks, whilst the limited sampling of these authors for Nd-Sr isotopic analysis of granitoids from a great variety of Precambrian and Phanerozoic environments cannot be regarded as adequate, in my view, to justify generalized global modelling of crust-mantle evolution. Every geological province, of whatever age, must be investigated in detail and treated as an individual case study, whilst isotopic data should not be discussed in isolation from geological, petrological and structural evidence in complex terrains. Initial Sr, Nd and Pb isotopic ratios of granitoid rocks are not directly dependent on their age but on their petrogenesis which, in turn, is closely related to the presence or absence of older sialic crust.

I have no argument with the overall isotopic conclusions of Clayburn *et al.*¹ that the granitoid rocks of the Etive Igneous Complex, ranging from calc-alkaline to granitic types, were produced as a result of melting of both contemporary mantle and lower continental crust, or with their interpretation that the process represents combined primary crustal growth coupled with assimilation of older stabilized continental crust. This type of situation is currently being modelled from many other igneous provinces. However, I ask these and other workers on crustal evolution to be specific and unambiguous in their use and interpretation of accepted terminology. Only by specifying precisely the investigated rock types can confusion and misunderstanding in the study of the great, polygenetic family of crust-forming granitoid rocks be avoided.

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PANKHURST REPLIES—We thank Moorbath for his comments on the introduction to our article¹ on the isotope geochemistry of the Etive Igneous Complex and apologize for any unintended misrepresentation of his view caused by a poor choice of words.

Our purpose was simply to set the scene by referring to two extreme schools of thought on the origin of granitoid rocks (s.l.) which have polarized around the older and necessarily restricted isotope data. Many workers have taken the primitive isotope characteristics of calc-alkaline granitoids (and sometimes true granites as well) to indicate primary crustal accretion solely through the processes of mantle melting and magma differentiation, despite warnings that a basic rather than ultrabasic immediate source for the magmas could be inferred from trace element data (see refs 2, 3).

We fully concur with Dr Moorbath in his criticisms of indiscriminate grouping of petrogenetically different granitoids by Allègre and Othman⁹, but, again, we were only concerned to draw attention to diverse views, however securely (or otherwise) founded. It remains of significance that our conclusions for the Etive Igneous Complex, which is of undoubtedly calc-alkaline parentage, recognize an appreciable (though difficult to quantify) component derived from much older lower crust and subjacent basic lithosphere as distinct from contemporary (Caledonian) mantle.

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Amines and secretory pathways

THE output of ACTH precursors from AtT-20 cells (a mouse pituitary line) has been shown by Moore *et al.*¹ to be enhanced by chloroquine (200 μ M). The cells normally synthesize a 30,000 molecular weight (30 K) precursor, from which they proteolytically cleave two forms of ACTH, 13 K and 4.5 K, which differ in their glycosylation. The precursor is externalized constitutively, as are other molecules such as a viral membrane glycoprotein, gp70. The cleaved products are mainly stored in secretory granules and discharged in the presence of a secretagogue, such as 8-bromo-cyclic AMP. Since chloroquine reduces storage of products, Moore *et al.* conclude that molecules of precursor are diverted from the secretory to the constitutive pathway. They suggest that ACTH packaging "may be another example in which a low intraorganelle pH is required for correct sorting of protein molecules". Chloroquine possibly "prevents the delivery of precursor ACTH to the secretory granule, which contains the specific protease", perhaps by elevation of the granule pH, as in packaging of lysosomal

enzymes^{2,3}. A general corollary of this idea is that it is the sorting mechanism itself which is disturbed. I wish to: (1) suggest alternatively that this mechanism *per se* is not modified, but that the concentrations of the substrates for sorting change; (2) point out the relevance of this to recent data in parallel systems⁴⁻⁶; and (3) sound a note of warning in the interpretation of such experiments with amines.

An alternative hypothesis for the causation of misdirection of ACTH precursor away from the secretory into the constitutive pathway, is that chloroquine inhibits the cleavages necessary for conversion of the 30 K precursor into the 4.5 K and 13 K products, and that consequently entry of substrate into the cleavage locale is feedback inhibited, allowing exaggerated traffic along the constitutive route, as a result of normal sorting.

There are several indications of this possibility in the data and discussion of ref. 1. First, the amount of newly synthesized ACTH and its precursors (that is all forms summed) recovered is enhanced by chloroquine (Table 1 of ref. 1). Second, less of the precursor is converted into the mature forms (Fig. 3 of ref. 1). These two observations indicate that proteolytic cleavage and degradation are retarded. Third, the ratio of the two cleaved mature forms in the cells is reversed by chloroquine (Fig. 3 of ref. 1), also suggesting an effect on cleavage independent of sorting. Fourth, the secretory granule enzyme(s) which can generate native ACTH forms from the precursors have acid pH optima, and the vesicles probably have an acidic interior¹. Finally, while the secretion of 4.5 K ACTH in response to secretagogue or in control conditions is inhibited by chloroquine (Fig. 1 of ref. 1), the secretion of 13 K ACTH product, like that of 30 K precursor, is not substantially elicited by the secretagogue, but is enhanced by chloroquine at least as much as is the secretion of the precursor forms (Fig. 2 of ref. 1): 13 K ACTH has thus presumably entered the constitutive pathway in both control and treated cultures. This suggests that the sorting mechanism is acting on the cleavage products rather than the precursors, and is functional in the chloroquine-treated cells.

We have made two observations which may also be explained by such an interpretation, especially if the relevant proteolytic cleavages include those on the intracellular degradative pathway which seems to be an alternative for many secretory proteins. First, plasminogen activator secretion by mouse macrophages is enhanced by another amine, ammonium chloride⁵. Second, the production by human monocytes of the apoprotein procoagulant thromboplastin, which is mainly transported to the cell surface (and not released), is similarly increased by a wide variety of amines⁶. The latter phenomenon is analogous to the enhance-

ment of output of gp70 by chloroquine (Fig. 2 of ref. 1). gp70 is measured in the medium, but this apparently reflects the amount transported to its normal site on the cell surface, from which it is released during the experimental protocol.

Finally, I stress that the effects of amines are complex, as the authors mention. In their work it is not necessary to postulate any effects of chloroquine on vesicle flux itself, as it may be merely the content of vesicles in the constitutive pathway which is affected (thus, increased 30 K precursor content (Fig. 3 of ref. 1) would result in greater 30 K secretion). But in other systems, inhibition of constitutive endocytosis^{7,10,11} and exocytosis by amines may involve changes in vesicle flux *per se*. Furthermore, the effects of a single amine, whether complex (chloroquine) or simple (NH₄Cl), often show biphasic dose-response curves. Thus, a multiplicity of effects may be involved in the present observations.

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MOORE ET AL. REPLY—We could not agree more with Dean that experiments that use amines to disrupt membrane traffic must be interpreted with great caution. We believe, however, that they can be of considerable value in dissecting membrane traffic patterns in cells when one pathway is perturbed by the amine while another is left essentially intact. Such was the case here¹ and for the lysosomal experiments².

Dean does not refute our conclusion that newly synthesized ACTH does not go to secretory granules in the presence of chloroquine, but is secreted constitutively. He suggests, however, that we are not correct in attributing it to an effect of chloroquine on the "sorting mechanism". The difference may be semantic. To us, a drug that blocks the generation of substrate for the regulated pathway, or its recognition by a specific receptor, or its delivery to the next compartment or the dissociation of substrates from the recep-

tor would still be an inhibitor of sorting. Dean prefers to equate sorting with the recognition step only.

A substantive issue raised by Dean concerns our conjecture that sorting precedes proteolytic cleavage of the ACTH precursor. We concede that the alternative hypothesis raised by Dean that proteolysis precedes sorting cannot be eliminated by currently available experimental data. Our reason for preferring sorting before proteolysis is in part a preference for simplicity. Since it is well established that the major products of the ACTH precursor, the NH₂-terminal fragment, ACTH and β -lipotropin, are secreted in equimolar amounts from AtT-20 cells³, Dean's hypothesis would require three sorting mechanisms for these three fragments, all working with approximately equivalent efficiency.

Second, if proteolysis precedes sorting there would be no point in packaging protease into secretory granules. In fact, the protease seems to be packaged with the ACTH into secretory granules, because (1) it is found there⁴ and (2) β -lipotropin is cleaved to β -endorphin long after packaging⁵.

Finally, although Dean's analysis of our results in terms of 13 K ACTH release is ingenious, there is proteolysis even of gp70 in the constitutive pathway and the higher ratio of glycosylated to non-glycosylated forms of ACTH results from a proportional increase in chloroquine in core glycosylation of all forms, perhaps because chloroquine slows passage of precursor ACTH through the Golgi apparatus.

We are also aware that chloroquine may be interfering with the crinophagic pathway and thus altering membrane traffic within the cell. Our data on this point are not, however, of sufficient quality to allow any exact conclusion. All we feel confident in maintaining is that the secretagogue-induced exocytosis is normal and the constitutive pathway is not inhibited at chloroquine concentrations at which there is a block between adding the endoglycosidase-H resistant sugars to ACTH precursor and the packaging into secretory granules. We do not know the nature of the block.

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Home truths around Jupiter

C.T. Russell

Physics of the Jovian Magnetosphere.

Edited by A.J. Dessler.

Cambridge University Press: 1983.

Pp. 540. £25, \$29.50.

EARLY in the era of space exploration we sent probes to the planets simply to discover what was there. Later, as the glamour of space exploration faded somewhat, it became fashionable (or perhaps necessary) to rationalize our quest to understand the planets in terms of improving our understanding of the Earth.

But how can you learn anything about the terrestrial magnetosphere from Jupiter? The Jovian magnetosphere has volcanoes in it, spewing out sulphur which becomes ionized and spun up by the rapid rotation of the planet. This rapidly rotating plasma stretches the Jovian field so that the magnetosphere is more disc-like than spherical. By contrast centrifugal force in the terrestrial plasma has an insignificant effect on the position of the magnetopause. Jupiter emits powerful radio waves, too, and releases energetic electrons which in some energy ranges dominate solar electrons at the Earth many AU away. In addition to this, it has radiation belts which roast our spacecraft if they linger too close to the planet.

We do learn of course. We learn basic plasma physics; we gain insight into how astrophysical plasmas may behave; we gain an appreciation of processes which operate on less grand scales in the terrestrial magnetosphere. And there are some processes that operate almost identically on the two planets, such as ELF and VLF emissions. On Earth, we cannot easily determine how much man's activity on the surface of the planet affects the generation of these emissions. The observations of Jupiter made so far indicate that these waves do, in fact, originate spontaneously in the plasma.

There is, however, still much to be learned, both from Jupiter and about it. Indeed the very structure of *The Physics of the Jovian Magnetosphere* reflects the immaturity of the discipline. There are eight observational papers centred around specific measurement techniques (magnetic fields, low energy particles, radio emissions, etc.) and four papers in which theorists discuss their favourite ideas. In a more mature phase of our understanding, such a book would have a series of articles discussing physical processes each of which covered all the relevant observations and theories. But we won't reach that stage for quite some time.

In the meantime this book will have to suffice. The authors have written sound, complete articles, not just the extended versions of oral presentations that you

often find in conference proceedings. These papers have been carefully reviewed and the contributors, perhaps with the editor's encouragement, have responded well. As a result the book is safe for first-year graduate students and others uninitiated to the sometimes intense rivalries that have developed as resources for such research have declined. The book would, however, have benefited from a discussion of satellite atmospheres and ionospheres (such a review is to be found in *The Satellites of Jupiter*, edited by D. Morrison and

published earlier this year by the University of Arizona Press).

The book ends with three appendices, a single list of references and an index. The first is a list of symbols and acronyms which was very handy in reading the book. The second, especially welcome, discusses coordinate systems; Dessler's jovial disposition is apparent in this attempt to prevent Jovian mispositions. The third appendix lists selected physical parameters of Jupiter and Io. Finally, there is the index, essential for any book of this type but all too often forgotten.

In short this is not a run-of-the-mill conference proceedings' volume, but a valuable thorough account of what we know — and don't know — about Jupiter's magnetosphere. □

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Venusian probes

Lionel Wilson

Venus.

Edited by D.M. Hunten, L. Colin, T.M.

Donahue and V.I. Moroz.

University of Arizona Press: 1983.

Pp. 1, 143. \$49.95.

CONSIDERING its similarity in size to the Earth and its obvious importance in understanding the origin and evolution of the silicate planets, it is particularly unfortunate that Venus is so well equipped (by its opaque, dense, hot atmosphere) to hide its surface from our probes. However, technological persistence pays off. To date, a total of thirteen Soviet and five American missions have visited Venus; the combined efforts have resulted in fifteen surface landers (some soft, some hard), seven flybys and three orbiters which has led to a steady increase in our knowledge of the planet.

A conference held in California in November 1981 provided the opportunity for a detailed review of Venus studies (see *Nature* 296, 13–20; 1982), and this book consists of much of the material presented at the meeting together with numerous papers submitted later by Soviet authors. As a result, the collection gives a well-balanced impression of the total effort being directed at Venus. It is interesting that a number of the papers are co-authored by Eastern-bloc and Western scientists, symbolic of an encouragingly high level of scientific collaboration. So great has been the upsurge of interest in Venus, particularly since the arrival in orbit of the US Pioneer Venus Orbiter in 1978, that the conference volume could not find room for all of the papers submitted — the interested

reader should also consult the special Venus issues of the journal *Icarus* dated August and November 1982.

A few of the papers in this volume are devoted to useful historical summaries; the rest are divided 14:6:5 among the atmosphere, ionosphere and surface, respectively. This emphasizes the leading role that studies of the atmosphere have played so far in the investigation of the evolution of Venus, and underlines the great amount of work remaining to be done on the chemical and physical structure of the lithosphere, the thermal state of the interior, and the nature of the tectonic regimes that represent the interaction between these two systems. Such studies are progressing steadily as a synthesis takes place between the surface chemical analyses made by the soft-landers Veneras 13 and 14 and the radar topography, roughness and dielectric constant data obtained by the Pioneer Venus Orbiter and by Earth-based radar measurements. However, one feels that it will be some time before our understanding of the surface and interior matches that of the atmosphere.

With the inclusion of a paper summarizing the Venera 13 and 14 measurements, made after the conference, this volume has the distinction of being a particularly up-to-date representation of our knowledge of Venus. However, two more Soviet probes are even now en route to the planet, and the US Venus Radar Mapper probe is expected to provide a very great increase in our knowledge of the surface when it begins measurements in mid-1988. So with respect to the surface, we can assume that the best is yet to come. □

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Filling in space

C.W. Kilmister

Relativity and Geometry.

By Roberto Torretti.

Pergamon: 1983. Pp.395. £22.50, \$45.

A PERENNIAL trouble with the philosophy of science, and, to slightly lesser extent, its history, is that of finding an adequate level of philosophical and historical scholarship in those trained fully to understand the science and vice versa. It is a joy to record how, in this "historico-critical exposition" of special and general relativity, Roberto Torretti triumphs over this problem.

The story begins with Newton, who is seen as the author of the revolution against Aristotle, but who also went wrong by assuming, in a natural seventeenth-century way, that featureless Euclidean space must be endowed with mechanical existence. This extra assumption then involved him in the need for an absolute space, just as he had already followed Aristotle in requiring an absolute time. Not that Newton had no choice for, since Elie Cartan in 1923, we can formulate mechanics against an appropriate Newtonian space-time. But the mathematical description of this involves such highly twentieth-century concepts that it is difficult to believe that even Newton could have done it had he wanted. (Of course, he did not want to, for he had sound theological reasons for preferring an absolute space.)

We move on to the familiar tale of electromagnetism and the aether, through Maxwell, to Einstein's 1905 paper which is dealt with at length. A thorough discussion is given, too, of Whittaker's mythical "Relativity Theory of Poincaré and Lorentz". The following chapter, just as long, on Minkowski space-time includes, as well as more familiar material, consideration of Robb's theory of a causal structure for space-time and also Zeeman's striking connection between the causal and the affine metric structures (that the group of causal automorphisms is the group of orthochronous similarities). An important role here is played by the concept of a causal space in the sense of Kronheimer and Penrose. Minkowski space-time is such a space, but Newtonian space-time fails to satisfy the requirement that any neighbourhood of a point contains a causal curve through the point. Robb's achievement was then to show that the same axioms that defined the causal structure of Minkowski space-time were sufficient to specify its affine and metric structures (up to a dilation).

Next there is a description of Einstein's search for a theory of gravitation, due attention being given to the curious papers of 1907 and 1911 (where a variable velocity of light serves as the gravitational poten-

tial). Here there is also a notably sympathetic treatment of Nordström's 1912 theory, which inspired Einstein and Fokker to reformulate it in 1914, and may perhaps have stimulated Einstein's physical thinking in just the way that was needed to allow him to appreciate the ideas of Marcel Grossmann. The description of general relativity goes beyond 1915 to discuss the problem of the equations of motion and the related one of gravitational radiation.

Torretti then proceeds to gravitational geometry, seeking to "elucidate a few matters that I consider especially significant from a philosophical point of view" — the role of the conformal structure of space-time, Mach's principle and cosmology, singularities and the main singularity theorems — before tackling what he calls "disputed questions". One of these is the definition of simultaneity (in special relativity), and, from that, the discussion of geometric conventionalism. Here Poincaré is not mentioned but Reichenbach is, getting short shrift for his misconstrual of gravity. Grünbaum, on the other hand, is castigated for obscurity. Finally questions of time and causality are discussed.

An appendix gives a modern, largely coordinate-free, description of manifolds, fibre bundles, linear connections, and there are also seventy pages of notes, only a small part of which is merely references.

It is clear that Torretti himself is equally at home when dealing with science, and with history and philosophy. The question then is who will constitute the audience for his book. Certainly any research worker in relativity will have his understanding of what he is doing (as distinguished from his mere technical know-how) greatly deepened by reading it. The author, however, naturally seeks to cast his net wider. He imagines a reader equipped with "a clear recollection" of n -variable calculus and of linear algebra and of classical mechanics and electrodynamics. (Little philosophical knowledge is called for because the author supplies it as he goes along.) Such people will find the book hard going, but correspondingly rewarding. □

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Upper crusts

Janet V. Watson

Mountain Building Processes.

Edited by Kenneth J. Hsü.

Academic: 1983. Pp.263. £39.40, \$72.50.

JUST another symposium volume on a plate tectonic theme? No, this book is different. The background seems quite familiar: a meeting in Zürich in 1981; a theme complex enough to arouse interest but not so vast as to seem formless; a couple of dozen contributors, neatly mixing the acknowledged masters with the young turks; and a dedicated editor who knows how to wheedle acceptable manuscripts out of his participants. Many useful but dull volumes have been built on just such a formula. What makes this one so enjoyable?

A clue is to be found in Professor Hsü's introduction, in which he uses the image of the anatomist reconstructing a living organism from a skeleton to illustrate the geologist's approach to mountain belts. The exhilaration which the authors have felt in breathing life into their subject comes through in article after article. A glance at the list of contributors — and at the photographs of Augusto Gansser and Rudolph Trümpy to whom the book is dedicated — shows that many of them are people with a lifetime's experience of mountains, people who have talked mountain-building in tents and cafés and landrovers all over the world. Moreover, they share a common interest in the interpretation of three-dimensional structures, an art fundamental to tectonic studies.

Another clue comes from the plan of the

volume as a whole, which is forward-looking but not trendy. The first part ("Processes") covers rock deformation, plate movements of many kinds, seismicity and magmatism. In the second part ("Case Histories") the effects of these processes are illustrated by reference to specific mountain belts, pride of place being given (fittingly, given the book's many Swiss links) to the Alpine-Himalayan belt.

Most of the articles are short and well-focused; little space is wasted on the details of what is already known and the emphasis falls all the more clearly on what is still debatable. The displaced terrains — those small slabs of continental crust which migrated for thousands of kilometres before being trapped in a collision zone — are discussed more than once. The still-unsolved problems of balancing structures observed in the upper crust against those inferred for the lower crust and lithospheric mantle are highlighted by a number of true-scale sections. Two excellent articles deal with the ductility of rocks and the mechanisms by which deformation takes place. There is a welcome lack of stereotyped "cartoons" and the vital but overworked arguments concerning crustal genesis have been given a well-earned rest.

The secret of success seems to be that the right authors have been asked to discuss the right questions. Collectively, they have produced a volume to which the general geologist can go not only for clear, pithy summaries of current problems but also for ideas and inspiration. It is a worthy tribute to two masters of European geology. □

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Describing a drug's charge

Gordon C.K. Roberts

Quantum Pharmacology, 2nd Edn.

By W.G. Richards

Butterworth: 1983. Pp.273. £25, \$59.95.

THE basic objective of molecular pharmacology was outlined by John Locke, in his *Essay Concerning Human Understanding* (1690):

Did we know the mechanical affections of the particles of rhubarb, hemlock, opium and a man, as a watchmaker does those of a watch, . . . we should be able to tell beforehand that rhubarb will purge, hemlock kill and opium make a man sleep . . .

Over the years, much effort has been devoted to investigating the "mechanical affections" which determine drug specificity; with the development of structural and molecular biology, the past thirty years in particular have seen signal advances in our understanding of drug action. Dr Richards's book (the second edition of a work first published in 1977) is concerned with the most recent phase of this continuing effort, in which the methods of theoretical chemistry are brought to bear on the attempt to relate specific structural features of the drug molecule to its biological effects.

The first part of the book (114 pages) provides a wide-ranging but unfortunately rather superficial outline of some basic pharmacology. This is designed as an introduction for theoretical chemists, but they would probably be better advised to refer to one of several good introductory texts which are available. The second part (51 pages), on the other hand, is an excellent and lucid introduction to

molecular orbital calculations which will be invaluable to any biologist venturing into this area for the first time.

In the final part (70 pages) we come to the heart of the book, where the applications of quantum chemistry to pharmacology are discussed; as one would expect, it is here that the greatest changes from the first edition are to be found. A substantial number of up-to-date examples are discussed in a helpful and reasonably critical way. These range from studies of drug conformation and of electron distribution and electrostatic potential, to correlations between activity and molecular orbital parameters. All this is presented very clearly, and one could only ask for more; additional references up to the end of 1981 are given in the excellent bibliography.

In this edition there is a new chapter on "Enzymes as Receptors", but only of eight pages in length. This area has been very active in recent years. However, much of the best of this work has largely involved "classical" potential functions rather than molecular orbital calculations, and these "classical" calculations are not discussed here. It is of course easy to castigate an author for not writing a different book, but a more rounded picture of the contributions of theoretical chemistry to pharmacology would have emerged if both kinds of calculation had been included.

Comparison of the first and second editions gives an impression of the steady progress in this field during the intervening years. It is clear that molecular orbital calculations now have an established place among the tools of the molecular pharmacologist, and Dr Richards's book continues to provide a useful introduction to their use. □

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Empowered to set standards

Alastair Hay

OSHA and the Politics of Health Regulation.

By David P. McCaffrey.

Plenum: 1982. Pp.192. \$29.40, £19.15.

WHO regulates the regulators? Is it industry or public pressure groups? Or does the bureaucracy in a government agency police itself?

The answer to these questions usually depends on your position. Industrialists might be expected to feel that pressure groups have too much influence, whereas public interest groups are often the first to accuse industry of using unfair lobbying tactics. Sandwiched in between are the officials in the regulatory agency itself. Do they allow themselves to be brow-beaten and take the easy way out, or do they genuinely seek an answer which is in the public interest?

Whatever the decision, it usually fails to please all concerned, as David McCaffrey points out in his review of decision-making in the US Occupational Safety and Health Administration (OSHA). In a remarkable and very readable book, McCaffrey analyses the decisions made by the agency in the ten-year period 1971-1981. But it is his approach to the issue which makes his book unique. Rather than merely documenting decisions and the reactions to them, he considers what the outcome would have been had industry, public pressure groups or bureaucratic self-interest arranged the outcome.

A comparative fledgling by agency standards, OSHA was born in the era of public concern over hazards in the work place. The Occupational Safety and Health Act of 1970 established the agency and charged it with ensuring that no worker should "suffer material impairment of health or functional capacity" from toxic chemicals at work.

Has it discharged that obligation? Only in part. There are some recognizable successes but many failures too. The reasons for this are not hard to find; with President Reagan anxious to "deregulate" industry the message to the regulatory agencies is a simple "hands off". And the appointment of people of like mind to head OSHA and the Environmental Protection Agency only helped to underline the message.

But such pressure was also evident under the Carter Administration, the imposition of cost-benefit analysis — lives saved versus cost to industry — doing more than anything to weaken the power of the regulatory agencies. For example, in 1977 the

Landmarks in science

Great Scientific Experiments: 20 Experiments that Changed our View of the World by Rom Harré (Phaidon Press), is now available in paperback from Oxford University Press; price £3.50.

The author considers key experiments in the history of science from a methodological and theoretical point of view. The work of Isaac Newton on the nature of colours is one of the subjects discussed. Below: The design of one of Newton's experiments, shown in the book.

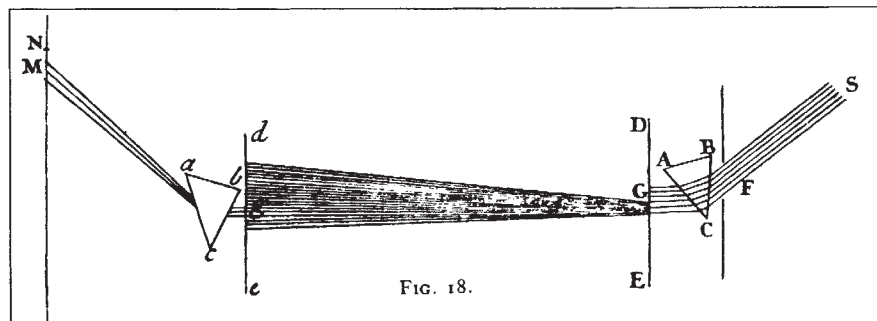


FIG. 18.

The separation of rays of different 'refrangibility'. (From *Opticks* 1721 edition, book I, part I, table iv, fig.18). S is the source of white light. In prism ABC the rays of different refrangibility are separated. The screens DE and de serve to separate progressively purer colours.

Council on Wage and Price Stability (CWPS) intervened when OSHA was considering exposure limits for cotton dust. Bowing to pressure from CWPS, the dust limit was eased by OSHA thus saving industry an estimated \$600 million annually; the price for this, the agency admitted, was about 5,260 cases of byssinosis.

By 1981 OSHA had issued only 21 complete health standards, covering some 16 carcinogenic chemicals as well as arsenic, lead, asbestos, cotton dust and coke oven emissions; its general carcinogens policy was — and still is — under discussion. Compulsory exposure limits had also been issued for some 400 substances but these were simply taken from earlier recommendations by the American Conference of Government Industrial Hygienists. Nonetheless opposition from industry to the vinyl chloride, coke oven, arsenic, cotton dust and lead standards was intense. The imposition was, says McCaffrey, “a significant accomplishment by OSHA”.

However, the battles in court over these standards have had their effect. According to McCaffrey, the agency now spends far more time in the preparation of regulations than it did in its formative years. Early court decisions, pointing out that there were so many uncertainties in the control of chemicals that decisions had to reflect policy judgements rather than a mere analysis of facts, have encouraged introspection to the point of timidity. Even the General Accounting Office — the investigatory arm of Congress — was moved to remark that decision-making in the agency was now taking far longer than it ought.

Standard-setting is also greatly influenced by the court which rules on the matter, the Fifth Circuit Court in New Orleans having the reputation of ruling in favour of industry and for less stringent controls; the US District Court for Columbia for supporting labour. Such differences result in some unseemly races to place the issue before a particular court. And a court ruling rarely settles the matter — often it is a stratagem adopted by industry to delay the imposition of controls.

McCaffrey is right to suggest that in the short term OSHA will not have many successes. But while he makes few predictions for the long term, the outlook may not be too bleak. Sooner or later the pendulum will swing back, giving OSHA some of the encouragement it so clearly needs to carry out the work Congress asked it to do. □

Alastair Hay is a Lecturer in the Department of Chemical Pathology, University of Leeds. He is author of The Chemical Scythe: Lessons of 2,4,5-T and Dioxin, published late last year.

• *Splendid Isolation: The Curious History of South American Mammals* by G.G. Simpson (Yale University Press), reviewed in *Nature* (286, 543), is now available in paperback; price £7.95, \$8.95.

Colour of success

Linda Partridge

The Arctic Skua: An Account of the Ecology, Genetics and Sociobiology of a Polymorphic Seabird.

By Peter O'Donald.

Cambridge University Press: 1983.

Pp.324. £25, \$49.50.

MANY important evolutionary questions can be answered only by demographic studies of populations of marked individuals. But partly for reasons of funding, studies of natural selection in long-lived species are very unusual. Peter O'Donald's book is a welcome addition to this area, the Arctic Skua (Parasitic Jaeger for North American readers) being fairly long-lived with an annual adult mortality of about 11%. The book is mainly an account of an eleven-year study of the bird on Fair Isle, with additional material on its general ecology and status.

The Arctic Skua's feature of particular interest is the presence of conspicuous discontinuous variation in plumage colour.

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Arctic Skua — pale morph.

There are three morphs (pale, intermediate and dark). While there is lingering uncertainty about the precise pattern of inheritance, it is clear that the different plumages are the result of a genetic polymorphism.

O'Donald's main interest is the process of sexual selection, and it is the role of this in maintaining the plumage polymorphism that he considers in detail. Sexual selection occurs because of variation in the quantity or quality of mates obtained by different individuals. Arctic Skuas are monogamous, so mate quality is the variable of interest. Among pairs breeding together for the first time (about 40% of breeders), those containing dark males breed earlier in the season and this early breeding is associated with high breeding success. O'Donald assumes that breeding date is determined by the female and that dark males are preferred as mates; when new pairs form the dark males acquire those females that are in breeding condition earliest and are thus able to breed at the most advantageous time. Sexual selection therefore favours dark males.

Sexual selection can occur in two ways. Intrasexual selection is a result of rivalry between members of one sex (usually the

male) for access to the other. Intersexual selection occurs where one sex chooses between potential mates. O'Donald attempts to show that dark male Arctic Skuas are preferred by females, and that this is why they obtain mates early in the breeding season.

However, the main evidence cited for female choice of male plumage is indirect only, relying on goodness of fit to models of the process, and it is a pity that the alternative mechanism of male competition was not more fully explored. Pairing takes place on territories defended by males and subsequently used for nesting and sometimes feeding. Dark males may acquire territories of better quality and females may choose territories rather than male phenotypes. Simple observations of territory establishment would help to clear up this question; one could also alter plumage or territory quality experimentally.

Sexual selection alone could not maintain the plumage polymorphism, although it might be expected to have led to the evolution of sex-limited plumage colour with all the males dark. Be that as it may, a further factor to be considered is that dark birds are at a disadvantage in that they first start breeding at four years or older, while pales may breed in their third year and hence are more likely to survive to breed at all.

At least on Fair Isle, the various selective forces are not in balance. Combining all the breeding data for males and females, and extrapolating to lifetime breeding success, pales have a net advantage. O'Donald concludes that the polymorphism is the result of geographically varying selective forces with migration between breeding colonies. There is a marked geographic cline in morph frequencies, with pales at highest frequency in the north of the distribution. Some as yet unidentified selective force must therefore give pale an advantage in the north and dark in the south of the species range.

It is a pity that O'Donald does not widen his discussion to other aspects of the bird's biology. It is difficult to collect data on skuas outside the breeding season, but there may well be differences in, for example, feeding ecology between the morphs.

Perhaps the greatest strength of the book is the rigour and clarity of the data analysis. Its main weakness is one often found in genetic studies, namely a disregard for the importance of direct observations of behaviour. The main conclusions do occasionally become buried in a mass of parameters and confidence limits, but the occasional withering attacks on earlier work add some spice. O'Donald's personal affection for the birds and their habitat comes through very clearly and, with the lovely Gillmor illustrations, compels the reader to share in his enthusiasm. □

Linda Partridge is a Lecturer in the Zoology Department at the University of Edinburgh.

This week's feature includes products ranging from scissors to flash arrestors, and from smoke generators to moisture meters.

● Brownell have announced a range of remote-reading dewpoint indicators which use the change in dielectric value of an anodic film, induced by changes in water vapour pressure, to indicate the equivalent dewpoint temperature of the vapour-bearing gas. The instruments have been developed to monitor the dewpoint level within electronic, electro-optical and optical equipment. They have a "preferred" power supply range of ± 5 V to ± 15 V, although the circuit can be arranged to operate on a variety of power supplies. Output signals to within 2 V of the top rail voltage will indicate dewpoints equal to or below the designated level. The indicators can be supplied for use as built-in test equipment where one designated level of dewpoint is indicated, or as maintenance indicators, with two or three levels of dewpoint which allow the user to plot the rate of degradation of the environmental conditions within the equipment. A further version gives an analog readout.

Circle No. 100 on Reader Service Card.

● The Isco 1580 waste-water sampler is now available in the UK from Protec. The model 1580 collects uniform small sample increments from streams and manholes at either timed or flow-proportional intervals. The sampling frequency can be continuously adjusted to any time between 2.5 and 320 min. Samples are under pumped flow at all times. The sampler is made of high impact plastic and has a 19-inch diameter circular construction for easy access to manholes. It can be powered either by the mains or a battery.

Circle No. 101 on Reader Service Card.

● The Oriel model 77500 quartz halogen fibre optic illuminator consists of a 100-W quartz halogen lamp with a built-in filter holder accepting 1-inch diameter filters. The transformer-powered illuminator features a separate light dimmer and a voltmeter to simplify reproducing voltage set-points. The model 77500 is the first of a series of four different quartz halogen sources designed specifically for fibre optic applications. The fibre optic cable-mount accepts glass or fused silica fibre optic cables, as well as high-transmission liquid guides.

Circle No. 102 on Reader Service Card.

These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Service Card bound inside the journal.

● To reduce particle and fibre generation in "clean" areas, Envirotech has introduced a new series of wipes based on a wet-laid, non-woven design that reduces linting figures by a factor of ten. The wipes consist of long, natural fibres on a manilla hemp base and are available in three different grades — 10C for delicate operations, 100K for general wiping and 17V for wrapping electronic components before assembly. Each grade is available in packs of 1,000 sheets, each 23 x 23 cm in size, and in roll form.

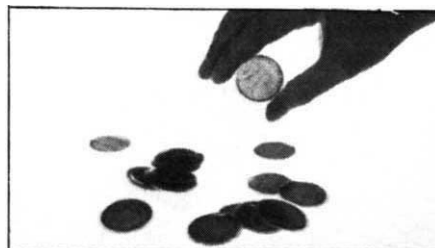
Circle No. 103 on Reader Service Card.

● To control the odour of high volumes of biohazardous waste in large autoclaves, Bel-Art Products have produced triple-strength Odo-Clave deodorant discs. Like the standard Odo-Clave deodorant discs, these are activated automatically during the heating cycle in the autoclave. They release a deodorizer which neutralizes offensive odours, leaving a mild residual scent. The Odo-Clave discs are supplied in packets of 100.

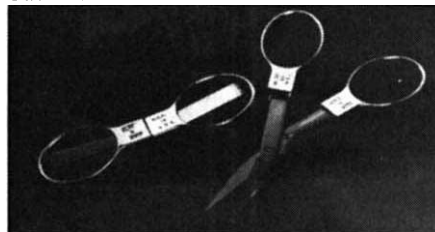
Circle No. 104 on Reader Service Card.

● B-J Scientific Products has introduced a compact folding scissor, called Slip'N'Snip, for use in the medical and scientific professions. These scissors have stainless surgical-steel blades and chrome-plated metal handles, and can cut through sheet metal with no damage to the blades. When not in use, Slip'N'Snip scissors can be folded up, with the blades shielded, and can be carried in a pocket, unlike standard scissors. Slip'N'Snip scissors come individually boxed with instructions.

Circle No. 105 on Reader Service Card.



Odo-Clave deodorant discs



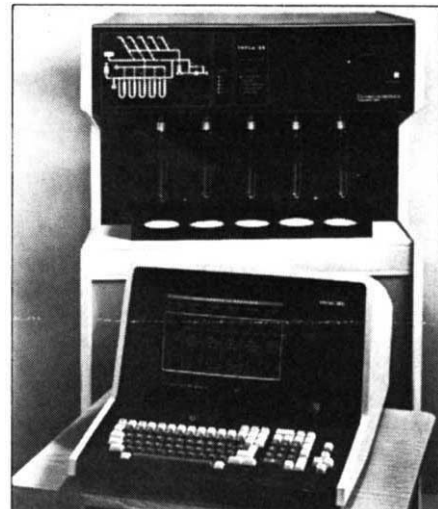
Slip'N'Snip scissors from B-J Scientific

● Anaspec has introduced universal lamp source assemblies for use with high aperture small or medium focal length monochromators. Four units are available: the 75 W xenon lamp assembly, the 100 W mercury lamp assembly, the 100 W quartz tungsten halogen assembly, and the 60 W deuterium lamp assembly. Each unit includes lenses to match the output of the lamp to the input aperture of f/3.5 to f/3.8 monochromators (such as the Jarrell-Ash 82-400 series). Each system has vertical and horizontal lamp position controls, and an adjustable condensing and focusing lens assembly to collect and then focus radiation on the slit of an f/4 type monochromator. Each also has a power supply and cables suited to its requirements. The lamp is supplied with a housing which ensures protection and controls the operating temperature.

Circle No. 106 on Reader Service Card.

● A new version of the Micromeritics ChemiSorb 2800 has been produced with more analysis options. The instrument provides automated chemisorption analyses designed to indicate how much of a catalyst surface is available to produce a desired chemical reaction. Called the Research Mode ChemiSorb 2800, the unit allows all phases of analysis to be individually programmed for each of up to five samples. New programming and the addition of a colour input/output module allows the automatic development of methods for catalyst sample preparation and analysis. Temperature, temperature-rate, time, gas (out-gassing) and pressure can all be varied.

Circle No. 107 on Reader Service Card.



The Micromeritics ChemiSorb 2800

● A new stainless-steel washing machine that cleans up to 300 laboratory animal cages in an hour is now available from New Smiths Stainless. The cages are carried through liquor jets, a soak tank and rinse jets on a horizontal closed-loop overhead conveyor. Throughput speed is variable to suit the condition of the cages and the quantity to be washed. The main components are made from stainless-steel to prevent corrosion, improve hygiene standards and reduce cleaning and maintenance costs. Running costs can be minimized by heating the machine's liquor with steam drawn from an existing boiler. Alternatively, an electric or gas water heater can be supplied.

Circle No. 108 on Reader Service Card.

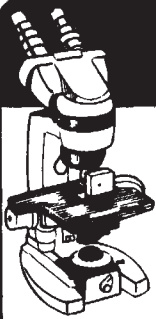
● A new range of Teflon valves and couplings is being marketed by Owens Polyscience. The new material from which the valves are made, Teflon PFA, can be injection-moulded. Components available range from needle, diaphragm, check and solenoid valves to stopcocks and union couplings. Physical ratings for the valves are from 90% vacuum to 125 p.s.i., and the valves will withstand temperatures of up to 150°C.

Circle No. 109 on Reader Service Card.

● To compliment their model CA-02, Anachem are now launching a new portable Coulometric moisture meter. This new instrument, the Mitsubishi model CA-10, can be run either off the mains or from a battery. It has a measuring range from 10 µg to 100 mg and a detection sensitivity of 1 µg water.

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Circle No. 18 on Reader Service Card.

● A new 300 µl microvette capillary blood collection system has been introduced by Sarstedt. The new microvette has an attached colour-coded stopper and a larger, funnel-shaped opening to facilitate the retrieval of collected blood. Microvettes are prepared with a full range of anticoagulants and are available with UV screening for bilirubin determinations. The new microvette is interchangeable with both the previous 300 µl microvette system and the 1.0 ml microvette size.

Circle No. 111 on Reader Service Card.

● A carbon-evaporation accessory — the CEA 010 — is now available from Balzers Union for use with their CTA 010 glow discharge unit. The new accessory enables carbon films to be both produced and conditioned within the unit. The CTA 010 glow discharge unit produces optimum surface properties for placing a specimen on a carbon support film. The film can be charged either positively or negatively, and can be made either hydrophilic or hydrophobic. The apparatus also has two vacuum chambers.

Circle No. 112 on Reader Service Card.

● A new air sampler has been designed by Envirotech. It monitors the presence of airborne particles and microorganisms, including asbestos, lead and other heavy metals, along with cotton fibres, ceramics, welding fumes, silica and toxic dusts. The unit is portable and can be run off a battery continuously for eight hours. During sampling, the air is drawn through a 25-mm membrane filter, although aerosol monitors of 37-mm diameter or 47-mm filter holders may be attached. In addition to the standard pore size of 0.8 µm used for monitoring airborne particles, pore sizes from 0.025 to 12 µm are available.

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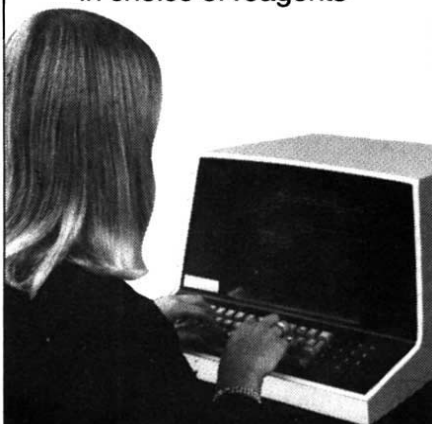
The CEA 010 carbon-evaporation accessory



The new air sampler from Envirotech

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Appointments

The British Committee of Vice-Chancellors and Principals has appointed **Mr Brian Taylor** secretary general of the committee. He is currently executive secretary.

The director general of the International Atomic Energy Agency (IAEA) has announced the following appointments: **Mr Carlos Buechler** has been made director of the division of standardization, training and administrative support, of the department of safeguards; **Mr Raymond Parsick** has been made director of the division of safeguards evaluation, of the department of safeguards; **Mr Vasilii Ferronsky** has been made director of the division of research and laboratories, of the department of research and isotopes and **Mr George Githii** has been made director of the division of publications, of the department of technical cooperation.

Dr Roger Iredale is to become principal education adviser to the Overseas Development Administration (ODA).

Mr Eric Bolton has been appointed senior chief inspector of Her Majesty's Inspectorate at the British Department of Education and Science. He succeeds Miss Sheila Browne who has resigned to take up the position of Principal of Newnham College, Cambridge, UK.

The new chairman of the Australian Atomic Energy Commission (AAEC) has been named as **Professor M.H. Brennan**. Professor Brennan will retain his current position of professor of plasma physics at the University of Sydney. Two new members of the commission have also been appointed. They are **Dr D.G. Walker**, the AAEC's chief executive officer, and **Dr J.G. Morris**, the director of the department of nuclear medicine at the Royal Prince Alfred Hospital, Sydney.

Mr Geoff Peters, senior lecturer in systems at the Open University, UK, has been elected dean of the Faculty of Technology.

Dr Timothy Carter has been appointed as the British Health and Safety Executive's director of medical services to fill the vacancy created by Dr K.P. Duncan's promotion to deputy director general.

The British Natural Environment Research Council (NERC) has appointed **Dr Brian Bayne** director of its Institute for Marine Environmental Research.

The British Medical Research Council

(MRC) has appointed **Dr Sally Macintyre** to be director of the council's medical sociology unit.

Dr Peter Horrocks has been appointed director of the National Health Service Health Advisory Service, UK.

Awards

On the recommendation of the Royal Society, Her Majesty the Queen has awarded Royal medals, also known as the Queen's Gold medals, to the following: **J.F.C. Kingman**, chairman of the Science and Engineering Research Council and professor of mathematics at the University of Oxford; **W.S. Feldberg**, professor emeritus and attached worker at the division of neurophysiology and neuropharmacology of the National Institute for Medical Research; and **D.J. Bradley**, professor of optical electronics at Trinity College, University of Dublin, Ireland.

The Royal Society, UK has made the following awards: the Copley medal has been given to **Professor R.R. Porter** for his work on the structure of immunoglobulins and the reactions involved in activating the complement system of proteins; the Davy medal to **Professor D. Arigoni** in recognition of his work in the fields of biosynthesis and bio-organic stereochemistry; the Hughes medal to **Professor J.C. Ward** for his contributions to quantum field theory; the Mullard award and medal to **Mr R.S. Hooper** and **Dr J.W. Fozard** for their contribution to the design, development and marketing of the Harrier V/STOL aircraft.

The Australian Royal Society of Victoria medal for scientific research for 1982 has been awarded to **Dr G.F. Mitchell**, head of the immunoparasitology unit at the Walter and Eliza Hall Institute of Medical Research, Australia.

Professor Robert Aumann and **Professor Philip Leder** have been awarded the 1983 Harvey Prize of the Technion-Israel Institute of Technology. Each will receive \$35,000.

Three prizes have been awarded by the General Motors Cancer Research Foundation for achievements in basic and clinical cancer research — the Kettering prize, the Mott prize and the Sloan prize. The Kettering prize, awarded for work in the diagnosis or treatment of cancer, has been given jointly to **Emil Frei III**, director of the Dana Farber Cancer Institute, USA and professor of medicine at Harvard School of Medicine, USA, and **Emil Freireich**, chairman of the department of

developmental therapeutics at the University of Texas System Cancer Center and professor of medicine at the University of Texas Medical School, USA. The Mott prize, given for work on the prevention of cancer, was won by **Bruce Ames**, professor of biochemistry at the University of California, USA. The Sloan prize, awarded for work in aetiology and pathogenesis, has been given to **Raymond Erikson**, professor of cellular and developmental biology at Harvard University, USA. Each prize is worth \$100,000, and award winners receive a gold medal and a grant of \$30,000 to hold a workshop in their specialist areas.

The Gairdner Foundation of Canada has announced its international awards for 1983. **Dr Donald Henderson** of the Johns Hopkins University, USA, has received an award of merit of \$25,000; and **Dr Bruce Ames** of the University of California, Berkeley, USA, **Dr Gerald Aurbach** of the National Institutes of Health, USA, **Dr John Clements** of the University of California, San Francisco, USA, **Dr Richard Gershon** of the Yale University School of Medicine, USA, and **Dr Susumu Tonegawa** of the Massachusetts Institute of Technology, USA have all received awards of \$15,000.

The winners of the Feldberg Foundation awards for 1983 are **Professor J. Aschoff** of the Max-Planck Institute for the Physiology of Behaviour, FRG and **Dr S. Brenner** of the Medical Research Council's Laboratory of Molecular Biology, Cambridge, UK.

The 1983 gold medal of the Ecological Society of Australia has been awarded to **Dr Leonard James Webb** for his investigations of Australian rain-forests and their relation to environmental factors.

George Bartholomew, professor of biology at the University of California, Los Angeles, USA, has been named as the first recipient of the Joseph Grinnell medal in vertebrate zoology, awarded by the Museum of Vertebrate Zoology of the University of California, Berkeley, USA.

Reginald Preston, emeritus professor in the department of biophysics at the University of Leeds, UK, has received the 1983 Anselme Payen award of the cellulose, paper and textile division of the American Chemical Society.

The trustees of the Lady Tata Memorial Trust have made international awards for research in leukaemia to: **R. Baer**; **A.P. Gee**; **K.M. Downs**; **A.G. Fisher**; **L.W. Kwak**; **D.D. Panjwani**; **U. Testa** and **M.G. Kauffman**.

Planned education or shortage of skills?

In the first of a monthly series of articles, Richard Pearson of the Institute of Manpower Studies outlines the case for planning the supply of skilled students to meet predicted demands.*

ALTHOUGH unemployment in Britain shows no sign of falling, complaints about the shortage of skilled recruits are getting louder. In information technology and microelectronics, employers cannot get the highly qualified staff they need, in biotechnology the "brain drain" is under the spotlight, and universities cannot fill their "new blood" appointments with the quality of staff they would like. In higher education, demand for places by would-be students is at its highest ever level, yet the number of places is being cut back. Can we identify the needs of a successful economy for skilled people and should higher education be geared to meeting those needs?

The fashionable view is that manpower planning does not work and therefore higher education should relate to social needs and student demands. In other

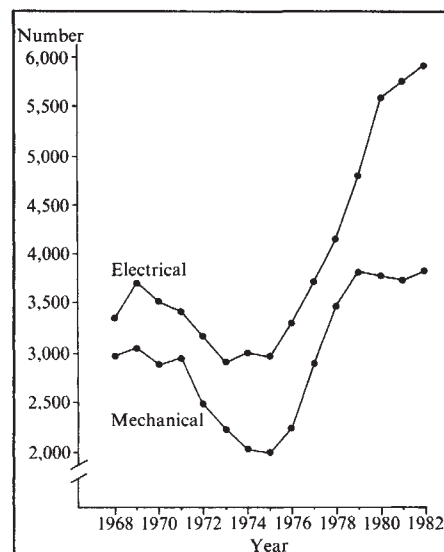


Fig. 1 Home applications to UK universities, 1968-82, for selected engineering subjects. Source: Universities Central Council on Admissions.

words, let the seventeen-year-olds determine the future supply of technical manpower and hence the rate at which we develop the new technologies.

Nevertheless, higher education is the route into most skilled technology-based jobs and clearly, with its huge costs, higher education cannot be divorced from economic reality. The recent cuts are forcing educational institutes to look outwards for finance and support. The government, through the "new blood" and "new

technology" initiatives, has provided extra, selective support, while the recent report from the Advisory Council for Applied Research and Development highlighted the need for more collaborative ventures. Do we need to be more positive about setting priorities? The answer must be "yes".

In setting priorities in higher education, we should be thinking of a selective group of key disciplines which will probably only account for perhaps one in three of the places. These should be our concern when seeking to relate education to economic or manpower needs. The remaining places can then be set according to social requirements, student demand or other criteria. Providing the places is, of course, not sufficient in itself. They need to be filled by suitable students, which in turn may require us to look at the funding of students, perhaps with differential grants, awards or sponsorships for key subject areas. It also means a stronger commitment by industry to demonstrate its belief in its future, by improving reward structures, and improving its "image" with students and educationalists.

The field of engineering is an example of the "cycle of mismatch" that can develop between supply and demand for skills. The early 1970s brought a cyclical recession; demand for newly-graduating engineers fell dramatically, and some companies were accused of withdrawing job offers; industry was being seen as undesirable and unreliable. Market signals "worked" and the level of application for engineering places fell dramatically (Fig. 1). By 1976 the "great debate" had been initiated, the Finniston report was in preparation, there was a dearth of good engineers, and industry began offering differential starting salaries for graduates, with premiums of up to £1,000 or more being paid for good engineers. Market signals "worked" again and applications began rising.

However, the consequences of the earlier fall were only then beginning to be felt. For example, with a significant decline in the numbers of students qualifying in electrical engineering (Fig. 2), the severe "shortages" of 1978-80 coincided with the low point in the supply cycle. Student numbers were, however, rising again and the output from universities in 1982 had finally recovered to the level of ten years earlier. Although demand has slackened since 1980, companies still have severe shortages of engineers with three to six

years experience, essentially those who applied to colleges in 1973-76 and graduated between 1977 and 1980. Thus we have a classic "cobweb" cycle of supply responding to market signals but being four years out of phase, with the negative signals being transmitted particularly quickly.

At least public policy over the 1970s was pushing in the right direction, with pressures to expand the number of places

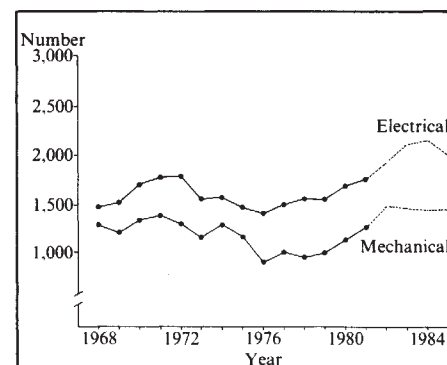


Fig. 2 First degree graduates (home students) from universities in 1968-85. Source: Institute of Manpower Studies projections.

and with innovations such as the enhanced degrees. The 1980s show us the consequences of our decentralized system of priorities. Notionally, engineering and technology were protected in the cuts. Yet with each institution relying on natural wastage and financial belt-tightening, electronics engineering, where costs are high and lecturers can easily find alternative jobs, has declining graduate numbers right up to 1985 (Fig. 2), a time when demand will be growing significantly if there is any marked economic recovery. Shortages will be back with a vengeance, strengthening the need to set national priorities. We need to invest now in manpower and training to meet our technological needs through to the year 2000 and beyond.

Defining these priorities will not be easy; we need better information, and links between employers and those planning higher education. At the same time, we need more flexibility within higher education to allow these priorities to be adjusted to changing needs. There will be costs involved, but the penalties of under-supply must be worse than those of oversupply.

From this perspective, I intend in the months that follow to deal with issues that include the extent and pattern of current shortages, the prospects for future employment and direction for public policy. □

*Institute of Manpower Studies, University of Sussex, Falmer, Brighton BN1 9RF, UK.